In the name of God
The Compassionate and The Merciful

The Innervation of Articular Blood Vessels

A Thesis submitted to the University of Glasgow
in candidature for the degree of
Doctor of Philosophy
in the faculty of medicine

by

Ali Khoshbaten B.Sc. (Cum Laude), M.Sc.

December 1989

Institute of Physiology
Faculty of Medicine
University of Glasgow

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

In the name of Allah
The Benefic ent, The Merciful

Who made good everything that he has created, and he began the creation of man from dust.

Then, He made his progency of an extract, of water held in light estimation.

Then, He made him complete and breathed into him of his spirit, and made for you the ears and the eyes and the hearts; little is it that you give thanks.

Holy Quran
Chapter 32
verses 7-9

Dedicated
to
my family

CONTENTS

Section III: Responses of articular blood vessels to acute joint inflammation in the rabbit.

CHAPTER FIVE

The innervation of knee joint blood vessels in the cat.

****

****

***

*

LIST of TABLES

LIST of FIGURES

Chapter 2: Following page

Chapter 3: Following page

Chapter 4: Section IA. Following page

Section III

Chapter 5: Following page

Acknowledgements

I am especially grateful to my supervisor, Dr. W. R. Ferrell of the Institute of Physiology, Glasgow University for giving me the opportunity and great support to pursue my studies under his keen supervision. The readiness and ease with which he offered suggestions to problems are really commendable. My study program in the Institute of Physiology was exciting mostly because of his constant interest in and support for my work, in adittion to his good humour. And again, I would like to give my special thanks to him for diligence and conscientious assistance in editing and clarification of the thesis.

To Professor S. Jennett for her advice and for giving me the opportunity to carry out this research in her department.

To Professor J. McGrath for his comentment and advice on some of the work in this thesis.

To Dr. R. Baxendale for his great welcome to me on my arrival in the Institute of Physiology and his help during my study.

To Miss Jess Wilson for her excellent technical assistance.

To my friends and colleagues in the Institute for being a great help to me, especially to A. Asgari for his help and encouragement during my experiments, and also to Dr. F. Lam for his help in using and calculation of Evans blue in these experiments.

To Department of Anatomy and mainly to my friends, M. Valogerdy and A. Hosseini in preparation of my histological works.

To Wilson Angerson for his help in counting the microspheres.

Last, but by no means least, and with my sincere gratitude, I wish to acknowledge the continued encouragement, and support of my parents, my wife and children during my academic studies.

Declaration and list of publications

The experimental work and other research which make up this thesis was carried out entirely by myself. No part of the material has previously been presented for any other degree.

Part of work contained in this thesis has been, or will be published as follows:

Abstracts

1). Khoshbaten, A. and Ferrell, W.R. (1989). Adrenoceptor profile of the rabbit knee joint blood vessels.The IX Iranian congress of Physiology and Pharmacology. Tehran - Iran.

2). Khoshbaten, A. and Ferrell, W.R. (1989). Dual action of ATP and role of endothelium in regulation of flow through blood vessels in the rabbit knee joint.The IX Iranian congress of Physiology and Pharmacology. Tehran - Iran.

3). Angerson, W. , Ferrell, W.R., Khoshbaten, A. (1989). Assessment of knee-joint blood flow in anaesthetised cats and rabbits. J. Physiol. (in press).

Papers

1). Ferrell, W.R. and Khoshbaten, A. (1989). Adrenoceptor profile of blood vessels in the knee joint of the rabbit. J. Physiol., 414, 377-383.

2). Khoshbaten, A. and Ferrell, W.R. (1989). Responses of blood vessels in the rabbit knee to acute joint inflammation. Annal. Rheu. Dis. (in press).

3). Ferrell, W.R.and Khoshbaten, A. (1989). The role of the endothelium in mediating the actions of ATP, Adenosine, and Acetylcholine on flow through blood vessels in the rabbit knee joint. British J. Pharmacol. (in press)

4). Ferrell, W.R.and Khoshbaten, A. (1989). Responses of blood vessels in the rabbit knee to electrical stimulation of the joint capsule. J. Physiol. (in press).

****

****

***

*

SUMMARY

Despite prevalence of inflammatory joint disease, at present little is known about the factors influencing articular blood flow. It was suggested that joint blood vessels in the dog are innervated by sympathetic efferent nerve fibres whose action is to constrict these vessels (Cobbold and Lewis 1956a). In other studies by Cobbold and Lewis (1956b), it was found that close intra-arterial injection of adrenaline and noradrenaline both produced vasoconstriction, but as to the types of adrenoceptors present on articular blood vessels, less is known. Dick and his colleagues concluded in their study on human that both α and β adrenoceptors were present on knee joint blood vessels (Dick et al 1971). There would appear, however, to be little information known about the nervous control of articular blood vessels either in normal subject or in diseased synovial tissue e.g. rheumatoid arthritis. The object of this research was firstly to assess suitable in-vitro and in-vivo techniques to study knee joint blood vessels along with measurement of blood flow in the knee joint capsule and surrounding tissues such as muscle and bones in both rabbits and cats. Secondly, to perform a more extensive and quantitative investigation in order to characterize the types of receptors that are distributed on articular blood vessels and are important in regulation of blood flow (e.g.

adrenoceptors, and purinoceptors), to identify the type(s) of nerves supplying joint blood vessels and their mediator(s), and whether the endothelium plays a role in regulation of these vessels. Attempts were also made to study changes in nervous control and receptors on these blood vessels that might appear in the acute inflammatory joint disease. Lastly, results obtained in the cat and the rabbit were compared.

Relative changes in blood flow were assessed by use of a perfused isolated knee preparation for the in-vitro studies, and both laser Doppler flowmetry and the microspheres technique were employed in the in-vivo experiments.

Results from this investigation indicate that 1). Perfusion technique and laser Doppler flowmetry methods provide suitable means of assessing relative changes in articular blood flow in-vitro and in-vivo respectively. 2). Blood vessels in bone around the knee joint of the cat and the rabbit are innervated by presumed sympathetic vasoconstrictor fibres travelling in nerves supplying the joint. 3). Post-synaptic α_1 , α_2 and pre-synaptic α_2 adrenoceptors but not β receptors are present on articular blood vessels. 4). Although P_1, P_2 purinergic receptors are present on vascular smooth muscle with P_2 receptor also present on the endothelial layer, almost all of the

data are consistent with the hypothesis that noradrenaline is the main neurotransmitter at knee joint blood vessels and produced vasoconstriction in response to articular nerve stimulation. It is also suggested that noradrenaline released from nerve ending affect mainly α_1 -adrenoceptors. 5). Afferent C fibres in articular nerves produce a dilator response to nerve stimulation and the mediator which is released from their terminals is most likely to be substance P. 6). Acute joint inflammation induced by kaolin increases the adrenoceptor sensitivity and influences those factors which normally regulate articular blood vessel calibre. The mechanisms that cause increase in sensitivity of adrenoceptors and the functional significance of this remain to be investigated. 7). The neurotransmitters released from nerves supplying the knee joint blood vessels and the receptors they act upon appear to be similar in both cat and rabbit. An interesting feature of these results is that, although the popliteal artery divides to give muscular and articular branches in close proximity to each other, the type of receptors on articular blood vessels are closer to that of superficial tissues such as skin than that of blood vessels supplying muscles. Thus, although both skeletal muscle and joints are considered to be deep structures, there is little homogeneity in distribution of receptors and types of innervation, which may reflect the differing function of these two vascular beds.

CHAPTER ONE

GENERAL
INTRODUCTION AND LITERATURE REVIEW

A: Introduction

I.Blood vessels

The peripheral circulation is essentially under dual control, 1) Centrally by the nervous system and 2) locally in the tissues by environmental conditions in the immediate vicinity of the blood vessels, with circulating hormones also influencing blood vessels calibre. The relative importance of these two is different in various organs and tissues. For example, in some parts of the body like skin and splanchnic regions, nervous control of blood flow predominates, whereas in others such as the heart and brain, neural regulation plays a minor role. But in general the fine control of blood vessels is brought about largely by competition between centrally directed vasoconstrictor nerves and vasodilator effects of locally produced metabolites.

The vessels chiefly involved in regulating the rate of blood flow throughout the body are referred to as the resistance vessels (small arteries and arterioles), since these blood vessels offer the greatest resistance to the flow of blood pumped by the heart and hence, are important in the maintenance of arterial blood pressure and also regulation of flow in different tissues and organs.

The smooth muscle of the resistance vessels is often spontaneously active , providing a "basal tone". The level of tone can be affected by extrinsic nerves and local factors. The phenomenon of autoregulation of flow (the increased vasodilatation that compensates for a primary decrease in pressure and flow through a circuit and the converse changes) is a product of the inherent myogenic tone of the resistance vessels.

A precapillary sphincter action regulates blood flow through the capillary network. This action is controlled primarily by local factors, but there is also an extrinsic nerve supply.

Changes in the human of the capacitance vessels (venules and veins) have a profound effect on venous capacity and hence on the filling of the heart and cardiac output , with little effect on resistance to flow . The smooth muscle of the capacitance vessels is controlled by extrinsic vasoconstrictor nerves. Apart from the spontaneous activity of the longitudinal muscle coat found in large veins , venous smooth muscle is generally quiescent.

Thus , in general, those components of the vascular circuit which subserve no local function, namely the large arteries and capacitance vessels , are controlled exclusively by extrinsic nerves, and show little spontaneous activity. On the other hand , the muscular arteries are under tonic neurogenic control of sympathetic noradrenergic vasoconstrictor fibres, which is governed by discharge from the medullary vasomotor centre. Decrease

discharge of these fibres produce a fall in tone of the vessels or vasodilatation without the involvement of specific vasodilator nerve fibres. In the precapillary resistance vessels vasodilatation is effected by release of various vasodilator metabolites. Catecholamines released from the adrenal medulla undoubtedly affects the smooth muscle of most vessels, but is of less general importance than neurogenic or local control of vessel diameter.

II. Anatomy of Autonomic Nervous System

The classical picture of the autonomic nervous system is that it consist of two major division , the sympathetic and parasympathetic systems.

The sympathetic nervous system consists of thoraco-lumbar outflow of preganglionic neurones passing via rami communicantes to make synaptic connection with postganglionic neurones in the paravertebral or prevertebral ganglia. The sympathetic chain , which consists of a bilateral system of paravertebral ganglia joined by longitudinal connectives , extends rostrally to the upper cervical region and caudally to the lower sacral level, but receives no efferent contribution from the spinal nerves at these extremes. Those postganglionic axons which pass out of the sympathetic chain to supply peripheral vascular beds and other superficial structure such as sweat glands pass via grey rami communicates to

the spinal nerves and run with them .

The parasympathetic system consists of portions of the outflows in the cranial (III , VII, IX, X, and XI) nerves and the sacral spinal nerve outflows. Preganglionic neurones from the central nervous system (CNS) make synaptic connections with postganglionic neurones in ganglia (e.g. ciliary ganglion) lying in or near the innervated organs.

The efferent fibres of the cranial division of the parasympathetic nervous system supply blood vessels of the viscera, whereas fibres of the sacral division supply blood vessels of the genitalia, bladder, large bowel, and uterus (Bell 1968). Skeletal muscle and skin do not receive parasympathetic innervation (Berne and Levy 1981). Thus only a small proportion of the resistance vessels of the body receive parasympathetic fibres.

In addition to sympathetic and parasympathetic efferent nerve fibres, there are some other fibres which arise from the motor cortex of the cerebrum and pass through the hypothalamus and the ventral medulla before joining the other sympathetic outflow in the spinal cord. This group is named the cholinergic sympathetic dilator system (Folkow et al 1961, Green and Kepchar 1959, Uvnas 1960). These fibres are activated in the "Alarm - Defence" reaction. There are also some reports that cholinergic sympathetic fibres can produce local vasodilatation of vessels in skin of the face and neck (Holton and Rand 1962, Hertzman 1959, Folkow 1955).

In general, autonomic fibres emerge in the ventral

roots of spinal nerves and in the ventral root of the first cranial segment (oculomotor nerve) while in the bladder region , autonomic fibres occur in nerves derived morphologicaly from dorsal roots (VII, IX, X Cranial nerves) (Goodrich 1930). Classically parasympathetic postganglionic neurones and cholinergic sympathetic efferent fibres release acetylcholine (ACh), whereas the antagonistic sympathetic nerves release noradrenaline (NA), (Berne and Levy 1981) Preganglionic fibres whether sympathetic or parasympathetic are mostly considered to be cholinergic (Dale 1931). Detailed descriptions of autonomic nervous system are avaliable in a number of text books (Kuntz 1953, Mitchell 1953, Berne and Levy 1981).

III. Aim of This Study

Synovial fluid plays a key role in diarthrodial joint function, lubricating the moving synovial and cartilaginous surfaces and supplying nutrients to much of the avascular articular cartilage. The factors affecting formation of synovial fluid have been considered in detail by Levick (1984). One of the important factors which determine the trans-synovial flow is the perfusion pressure across the synovial vascular bed (Knight and Levick 1984). However, relatively little is known about the factors influencing flow through articular blood vessels. As Liew and Dick (1981) reported, the lack of appropriate methods of study could be the reason.

Therefore, the object of the present study was firstly to assess suitable techniques to study knee joint blood vessels either in-vitro or in-vivo. Secondly;

a. To perform a more extensive and quantitative investigation in order to characterize the types of known receptors which are distributed on blood vessels and are important in regulation of blood flow (e.g. adrenoceptors, purinoceptors, cholinergic and histaminergic receptors).

b. The innervation of joints was reviewed by Gardner (1950) but he pointed out "The manner in which articular vessels are controlled , however, remains to be studied". In a few experiments by Cobbold and Lewis (1956), they showed that , like other blood vessels, articular blood vessels are supplied with sympathetic vasoconstrictor fibres through which they are maintained in a condition of tonic constriction. However they did not have the agonists and antagonists of adrenoceptor to characterize the types and subtypes of adrenoceptors. The purpose of this research was to identify the types of receptors and the type(s) and nature of neurotransmitter release from nerve ending on articular blood vessels.

c. In 1981 Furchgott described the role of the endothelium in control of blood flow in many blood vessels, hence in present study, this role has been investigated in some aspects.

d. In the present investigation the responses to electrical stimulation of the nerve supply to the rabbit knee joint were examined during experimentally induced acute joint inflammation. This was performed to find out whether the vasodilatation known to occur in joint inflammation could be attributed to alteration of neurotransmitter release at sympathetic nerve endings or change in adrenoceptor sensitivity induced by the inflammatory process.

e. Finally, another purpose of the present study was to attempt to compare some of the results between two different species (rabbit and cat).

B: Literature Review

Almost all smooth muscle receives innervation from the autonomic nervous system. The innervation may be excitatory in that it makes the muscle contract, or it may be inhibitory and prevent contraction or cause relaxation.

I. Adrenergic Nervous System

In general, sympathetic adrenergic nerves innervate most of vessels of the body, although in varying degrees. The degree of innervation ranges from sparse in the cerebral vessels to dense in skin. The density of innervation of the skeletal muscles and gastrointestinal tract is intermediate between the extremes represented by the cerebral and the skin vessels. The large arteries and veins are sparsely supplied whereas small arterioles are profusely innervated.

Concept of adrenergic receptors

Most drugs exert their potent and specific effects on the tissues by forming a bond , generally reversible, with some cellular constituent. This cellular constituent is the receptor.

The role of the receptor is to recognise a chemical signal and to discriminate between such a signal and other molecules. The receptor concept was first proposed by Langley in 1878. The presence of receptors at an anatomical site determines the selective nature of many drug effects.

One of the main roles of receptors is where the autonomic nervous system makes synapses with actual tissues (e.g. vascular smooth muscle), and neurotransmitters are released from nerve ending after passing through the synaptic cleft, and bind with the receptor either on the post-synaptic or pre-synaptic membrane.

The chemical neurotransmission theory has been proposed since the begining of the twentieth century and became the second theory of neurotransmission beside the electrical transmission.

In 1905 Langley showed that, there is similarity between the rise in blood pressure which was noted when adrenal extract was injected in-vivo (Oliver and Schafer 1894) and pressor response obtained by stimulation of sympathetic nerves. This rise in blood pressure remained after denervation, indicating a site of action on the effector system and not via the nerves.

The chemical which was responsible for the pressor action of the adrenal extract was termed adrenaline because of its close association with the adrenal medulla.

In 1905, Elliott was the first to suggest that adrenaline was liberated from the sympathetic nerve

endings when they were stimulated and that the released adrenaline then acted on the responsive cells (Elliott 1905) .Barger and Dale tried different compounds related to adrenaline, and pointed out that noradrenaline more closely mimicked the effect of sympathetic stimulation (Barger and Dale 1910).

The first conclusive evidence that chemical transmission occurs during nerve stimulation was obtained by Loewi in 1921. He set up two frog hearts and perfused them in such a way that the perfusate flowed from the first frog heart to the second one. When he stimulated the sympathetic nerves innervating the first heart, he observed that heart beat and contractility of both hearts had been increased. So he concluded that the nerve endings in the first heart were liberating a substance which he named "Acceleranstoff" (Loewi 1921), and that this substance was transported via the perfusate to cause the observed effects on the second heart. The striking similarity between the action of acceleranstoff and that of adrenaline on frog tissue led to investigations which eventually proved adrenaline to be the sympathetic transmitter in the frog.

However, in mammals the sympathetic neurotransmitter was shown to be noradrenaline (NA) rather than adrenaline (Table 1). Von Euler in 1946 showed that the main sympathetic transmitter was NA since very little adrenaline was present in sympathetically innervated tissue whereas the concentration of NA was very high by comparison. He also showed a decrease in NA levels

TABLE 1

Animal	Tissue	Assay Methode	Noradrenaline ug/g	Adrenaline ug/g	References
Guinea-pig	Heart	Bio and fluorometric	1.80	0.20	Antone & Sayre 1962
Bat	Whole heart	Fluorometric	0.92, 0.88		Nielsen & Owman 1968
Ground Squirrel	Whole heart	Fluorometric	1.51, 1.03		" "
Hedgehog	Whole heart	Fluorometric	1.18 ± 0.07		" "
Mouse	Heart	Bio and fluorometric	0.45	0.10	Antone & Sayre 1962
Albino Mouse	Whole heart	Fluorometric	0.65 ± 0.05		Nielsen & Owman 1968
Rat	Heart	Fluorometric	0.27	0.05	Antone & Sayre 1962
	Heart		0.60		Hokfelt 1951
	Heart		0.5		Leduc 1961
Albino rat	Atrium	Fluorometric	1.19 ± 0.20		Nielsen & Owman 1968
	Ventricle		0.46 ± 0.06		
Dog	Heart	Bio and Fluorometric	1.01	0.11	Antone & Sayre 1962
Rabbit	Heart		0.17-0.54	0.045-0.094	Holtz,et al 1951
Sheep	Heart	Bio and fluorometric	1.05	0.17	Antone & Sayre 1962
	Heart		0.6-1.1		Laurent 1962
Cow	Heart	Fluorometric	0.3-0.6		Laurent 1962
	Arteries and Veins		0.3-0.5		
Cat	Heart		0.5-1.0		Euler 1956
Cat	Atrium	Fluorometric	1.20 ± 0.11		Nielsen & Owman 1968
	Ventricle		1.52 ± 0.08		
Man	Heart	Bio and fluorometric	1.04	0.18	Antone & Sayre 1962

Table 1: Distribution of Noradrenaline and Adrenaline in Heart (From: Burnstock 1969)

following sympathectomy of the tissues, indicating NA association with nerve rather than muscle.

In 1948 Ahlquist identified two kind of adrenergic receptors based on the relative potency of adrenaline and NA, and isoproterenol to stimulate the receptors and on the ability of Dibenzyline (Phenoxybenzamine hydrochloride) and Dichloroisoproterenol (DCI) to block their effects (Ahlquist 1948). Ahlquist found that adrenaline was the most potent stimulator for one receptor, called the alpha (α)type, whereas isoproterenol was the least potent. Isoproterenol was the most potent stimulator for the other receptor, called the beta (β) type, and NA was the least potent. Phenoxybenzamine and phentolamine selectively blocked the action of the alpha-receptors, and DCI selectively blocked the beta-receptors. This differentiation of alpha and beta adrenergic receptors is summarized in table 2.

The adrenergic receptors differ in their physical and chemical properties to such an extent that their affinities for various adrenergic agents also differ. In general, the effect of the alpha-adrenergic receptors are excitatory and that of the beta-adrenoceptors are inhibitory. One important exception is beta receptors in the myocardium, which are excitatory in nature. Isoproterenol, by activating the myocardial beta-receptors, increases heart rate and force of contraction.

This theory was further strenghtened by the use of DCI by others (Powell and Slater 1958). This drug was

TABLE 2

Table 2: Differentiation of adrenergic receptors (alpha and beta).

Type of receptor	relative potency of			blocked by	
	Adrenaline	Noradrenaline	Isoproterenol	Dibenzyline[1]	DCI[2]
Alpha	500	100	1	+	-
Beta	20	1	100	-	+

1. Dibenzyline= Phenoxybenzamine hydrochloride
2. DCI= Dichloro-isoproterenol
(From cardiovascular and pulmonary physiology; Green 1982)

chosen as a selective inhibitor of beta receptors. Thus the terminology of alpha and beta receptors became accepted and lies at the basis of adrenergic neurotransmission research.

In 1967 Lands and his co-workers found that using sympathomimetic amines (beta-agonists) on both isolated tissues and whole animals lead to different responses. So he and his colleagues showed that there were two different types of beta-receptors, termed as $beta_1$ and $beta_2$ (Lands et al 1967). Up until 1972 it was widely believed that there were two subtypes for beta receptors, but no subtype for alpha receptors (Furchgott 1972).

So far, the receptors which were discovered were located on the effector muscle. However the discovery of pre-synaptic alpha-adrenoceptors followed the multiple actions of the alpha-adrenoceptor blocking agent phenoxybenzamine and led to the pharmacological subdivision of alpha receptors (Brown and Gillespie 1957). Presynaptic alpha-adrenoceptors have a different mode of action as they do not produce direct contraction or relaxation of smooth muscle but mediate inhibition of transmitter release from sympathetic nerve ending via a negative feedback mechanism (Langer 1974, Westfall 1977, Vizi 1979). Langer (1974) proposed that pre- and post-synaptic alpha receptors were not identical. He suggested that post-synaptic receptors to be named as $alpha_1$ and presynaptic receptors as $alpha_2$. Following the definition by Langer (1974), studies on relative potencies of different alpha agonists and antagonists at the pre-

and post-junctional sites were carried out.

Starke et al (1975a) were the first to report that the drug yohimbine displayed a preferential blockade of the presynaptic alpha receptor as opposed to the post synaptic receptor, in the rabbit main pulmonary artery. Other studies using agonists showed that alpha agonists varied widely in their pre- and post-synaptic potencies (Starke et al 1975b,c). In the rabbit pulmonary artery, methoximine was the most potent agonist tested at the post-synaptic receptors, whereas oxymetazoline was the most potent pre-synaptically. They concluded that it may be due to a structural differences in the two subtypes. Just as yohimbine has been introduced as a selective $alpha_2$ antagonist, prazosin was discovered to be a selective $alpha_1$ antagonist (Cambridge et al 1977).

Therefore, by using different agonists and antagonists potencies, in time it has been possible to identify and classify the subtypes of alpha-adrenoceptors in cardiovascular system (CVS), and elsewhere.

Moulds and Jauernig in 1977 discovered that prazosin act as a competitive antagonist at alpha-receptors but had selective effects on different vascular beds (e.g. peripheral beds such as the palmar digital artery were resistant to prazosin).

It was Docherty and McGrath (1980) who finally concluded that there are two kinds of post-synaptic alpha adrenergic receptors on vascular beds named $alpha_1$ and $alpha_2$ sub-types (Docherty et al 1979, Docherty and McGrath 1980). The classification of the post-synaptic

alpha$_2$-receptor was much aided by the discovery of a more specific and potent selective alpha$_2$ antagonist in rauwolscine (Weitzell et al 1979).

Since then, vascular post-junctional alpha$_2$-adrenoceptors have been clearly demonstrated in many animal in-vivo preparations(for reviews see McGrath 1981,1982,1983, Timmermans and Van Zwieten 1981,1982).

There is evidence that, even all α_1-adrenergic receptors are not pharmacologically homogenous. In a recent review article two subtypes of α_1 were also distinguished as α_{1a} and α_{1b} (Minniman 1988).

In summary, the classification of adrenergic receptors are two types alpha and beta receptors which these two are subdivided to two sub-types alpha$_1$ and alpha$_2$ adrenoceptors and beta$_1$ and beta$_2$ sub-types. Alpha$_2$-adrenoceptors are located either on pre- or post-synaptic membrane, whereas alpha$_1$ and beta receptors are known to be on post-synaptic membrane. Alpha$_1$ is itself divided to α_{1a} and α_{1b}.

II. Cholinergic Nervous System

In 1898 Langley noted that stimulation of the vagus nerves could produce relaxation of the stomach. This relaxation was best revealed after blockade of the excitatory action of cholinergic fibres with atropine (May 1904, Mc Swiney 1929). In this early work, it was usually assumed that the inhibitory responses were due to

nerves of sympathetic origin running in the vagal trunks. Later, it was shown that inhibition was due largely, if not entirely, to fibres of parasympathetic origin, since relaxation of the smooth muscle of stomach produced by stimulation of autonomic centres in the brain was mediated by the vagus nerve itself (Eliasson 1952, 1954, Hesser and Perret 1960, Semba et al 1964).

The first hint that some of the inhibitory fibres to the vertebrate stomach were not adrenergic appeared when adrenergic neurone blocking drugs were used. The inhibitory response of the guinea-pig stomach to vagus nerve stimulation was not prevented by these drugs (Greeff et al 1962).

Although vasodilator fibres have a limited distribution to the systemic circulation they can have a profound physiological influence under special circumstances. These vascular beds are known to recieve dilator innervation mediated by a nonadrenergic transmitter.

There are two basic types of vasodilator fibres 1) Cholinergic sympathetic nerves supply only the larger precapillary arterial vessels of skeletal muscles and are activated by a defense-like reaction (Green 1982). 2) Parasympathetic nerves which are of two types: a, those that produce dilation indirectly (Uvnas 1954, Beck and Brody 1961, Zimmerman 1968). b, There are those act directly on blood vessels to induce vasodilatation(Berne and Levy 1981).

Concept of Cholinergic Receptors

A partial isolation of the cholinergic receptor has been achieved by Hubbard and Quastel in 1973, There are muscarinic and nicotinic receptors known for acetylcholine which is neurotransmitter released from cholinergic nerve endings. Muscarinic receptors are present in various smooth muscle, cardiac muscle, and exocrine glands. They were termed muscarinic because muscarine , a quaternary amine alkaloid, has actions similar to those of ACh at the site indicated. The musicarinic receptor is competitively blocked by atropine and related drugs.

The nicotinic receptors of ACh are located in autonomic ganglia and at skeletal neuromuscular junctions. They are termed nicotinic because nicotine also acts on these receptors. The nicotinic receptors in autonomic ganglia and in skeletal muscle are not identical. The receptors in autonomic ganglia are blocked by hexamethonium, whereas the receptors at the skeletal neuro-muscular junction are blocked by d-tubocurarine and related compounds (Goth 1981).

For many years it has been something of a pharmacological paradox that acetylcholine (ACh), acting via muscarinic (Atropine-sensitive) receptors, is a very potent contracting agent for many kinds of nonvascular smooth muscle but a very potent vasodilatory agent for most of peripheral vascular beds. In an accidental finding in Furchgott's laboratory, after adding carbachol

to a aorta ring that was already contracted by noradrenaline, the ring partially relaxed (Furchgott 1981). Within a few weeks of this accidental finding which was unexpected, Furchgott and his colleques had established that the lack of relaxing response to Ach in other preparation such as the helical strip was the result of unintentional rubbing of endothelial layer against foreign surfaces (including finger tips). Thereafter the existence of cholinergic receptors on endothelium and their role in many blood vessels were realised.

III. Non-adrenergic, Non-cholinergic Nervous System

It seems that the first hint of a non-adrenergic, non-cholinergic system (NANC) came from the work of Langley and his colleagues at the end of nineteenth century. "Atropine-resistant" excitation of the bladder in response to stimulation of pelvic nerve was recognised (Langley and Anderson 1895), and few years later, inhibition of the stomach during vagal nerve stimulation was revealed following the block of cholinergic excitation by atropine (Langley 1898). Further hints were reported during the next 60 years (Mc swiney and Robson 1929, Paton and Vane 1963). However, these findings were interpreted in terms of different types of adrenergic nerves or inaccessibility of the cholinergic antagonist to the neuromuscular junction.

By the end of the 1960's the NANC system was

recognised by Burnstock and colleagues following various experiments in their laboratory (Burnstock et al 1963,1964, Burnstock 1969). They concluded the existence of a third autonomic nervous system not only in gastro-intestinal tract of all vertebrates including man but also in the urogenital, respiratory and cardiovascular systems.

Clearly it was important to name this third autonomic nervous system according to the kind of neurotransmitter(s) that are utilised and released. Systematic studies were carried out in the late 1960's and early 1970's to find out the substance(s).

Many substances were explored as possible transmitters mediating the responses to stimulation of the NANC nerves (Table 3).

In 1970 Burnstock and his colleagues concluded that amongst all substances, ATP is the most acceptable substance for the NANC transmitter in intestine and bladder (Burnstock 1970). and the Purinergic nerve hypothesis was formulated (Burnstock 1972).

Since then considerable evidence has accumulated in support of this hypothesis, although there have also been several reports that oppose it (Burnstock 1979,1981, Gillespie 1982). In general the evidence for purinergic NANC neuro-muscular transmission in the gut, bladder and rabbit portal vein is strong, but in some other organs, particulary those in interneurones, utilise different transmitters.

Knowledge of purinoceptors is one of the important

TABLE 3

Adenosine triphosphate	ATP
5-Hydroxytryptamine	5-HT
Gama-Aminobutyric acid	GABA
Dopamine	DA
Peptides	
Enkephaline/Endorphin	Enk/End
Vasoactive intestinal polypeptide/ Peptide HI	VIP/PHI
Substance P	SP
Gastrin releasing peptide/Bombesin	GRP/BN
Somatostatin	ST
Neurotensin	NT
Luteinizing hormone releasing hormone	LHRH
Cholecystokinin/Gastrin	CCK/G
Neuropeptide Y/Pancreatic polypeptide	NPY/PP
Galanin	GAL
Angiotensin	AII
Adrenocorticotrophic hormone	ACTH
Calcitonin gene-related peptide	CGRP

Table 3: Putative non-adrenergic, non-cholinergic transmitters (from Burnstock 1986)

aspects of purinergic transmission which help the hypothesis of purinergic NANC transmission. In late 1970's, Burnstock proposed two major types of purinoceptor (Burnstock 1978), P_1-purinoceptors which are most sensitive to adenosine are competitively blocked by methylxanthines and whose occupation leads to changes in cyclic AMP accumulation. P_2-purinoceptors are most sensitive to ATP, are not blocked by methylxanthines or act via an adenylate cyclase system and occupation leads to production of prostaglandins. Since 1978, subclasses of both P_1- and P_2- purinoceptors have been also proposed (Burnstock 1981b, Burnstock and Kennedy 1985).

IV. Neurotransmitters and related substance

A neurotransmitter is a substance which is synthetized and stored in nerves, and released by exocytosis during nerve activity from its vesicular storage site in the nerve terminal to diffuse across the junctional cleft to occupy specific receptors on the post junctional membrane, leading changes in postsynaptic activity.

Until recently it was widely believed that each neuron makes and releases only one transmitter. This is known as Dale's principle. At first this was based on chemical transmission in two nervous system such as adrenergic nerves releasing a catecholamine (subsequently identified as NA), and cholinergic neurones releasing Ach.

But since the 1970's there has been growing recognition of the plurality of transmission mechanisms. A variety of further substance are now claimed as established or putative neurotransmitters (Table 3).

In 1976, Burnstock wrote an article which was entitled " Do some nerve cells release more than one transmitter?". Today, co-transmission is widely accepted and it would be difficult to make a case that any nerve fibre contained only one transmitter (Burnstock 1986). Evidence has been presented for co-existence and release of ATP and NA from sympathetic nerves supplying the vas deferens (Westfall et al 1978, Burnstock 1983, Sneddon and Burnstock 1984a), and several blood vessels (Su 1975, 1983, Muramatsu et al 1981, Sneddon and Burnstock 1984b, Hicks et al 1985). Sympathetic transmission involving NA, ACh, and purinergic transmitter has also been demonstrated in cultured sympathetic nerves forming junctions with heart muscle cells (Potter et al 1983).

a. Catecholamines

A comparison of the level of noradrenaline (NA) and adrenaline (AD) found in the heart and arteries of different mammals show that in general the predominant catecholamines in sympatheticaly innervated tissues is NA (Table 1). This distribution appears to be the reverse of the proportions of NA and AD contained in adrenal gland (Table 4).

Apart from NA and AD, high levels of dopamine have

TABLE 4

Animal	Assay Method	Noradrenaline ug/g	Adrenaline ug/g
Guinea-pig	Bio and Fluo-rometric	2.50	84.93
Mouse		96.60	320.00
Rat		172.00	600.00
Dog		270.94	289.68
Sheep		296.90	866.10
Man		86.79	1173.20

Table 4: Distribution of Noradrenaline and Adrenaline in adrenal gland (Antone and Sayre 1962).

been found in the pacemaker region (sinus venosus) of the frog heart, and it has been suggested that dopaminergic nerve fibres may supply this region of the heart (Angelakos et al 1965). Similarly, high levels of dopamine have been found in the sino-auricular node in mammals (Angelakos 1963, Angelakos et al 1965).

Although dopamine acts on cardiac beta receptors and also on vascular alpha receptors (in large dose), some of its vasodilator effect suggest the exsistence of specific dopamine receptors (Goldberg 1974). Dopamine produces vasodilatation in the renal, mesentric, coronary, and intracerebral arteries. This action is not antagonized by propranolol but is selectively attenuated by haloperidol and phenothiazines (dopaminergic blockers).

b. Acetylcholine(Ach)

High levels of choline acetylase, acetylcholine and cholinestrase have been reported in visceral organs of all vertebrate classes (Augustinsson 1948, Feldberg 1945), and Ach has been established as a transmitter substance in the autonomic system of many species (Nelemans 1951, Grundfest 1957) and specially in some blood vessels Ach is known to be the transmitter responsible for the sympathetic vasodilatation in the skeletal muscle and skin of several species (Uvnas 1954, Beck and Brody 1961, Zimmerman 1968).

c. Adenine Nucleotides

ATP, is a transmitter proposed for non-adrenergic, non-cholinergic (purinergic) nerves in the vertebrate gastro-intestinal tract and probably other organs (Burnstock 1969,1972,1975), and it seems likely that some ATP is released together with NA from adrenergic nerves (Su, Bevan, and Burnstock 1971).

V. Coexistance of neurotransmitters

The suggestion that some nerve cells store and release more than one neurotransmitter was made in 1976 (Burnstock 1976) largely on the basis of comparative studies of the evolution of the autonomic nervous system (Burnstock 1969), and evidence for the coexistence of biologically active substances in certain invertebrates nerves (Brownstein et al 1974).

There is compelling evidence that under certain conditions <u>in vitro</u> single sympathetic neurons may release NA, Ach or a mixture of these two transmitter substances (Bunge et al 1978, Furshpan et al 1976). A detailed account of the evidence for coexistence of Ach and NA with ATP is available (Burnstock 1982).

The ACh:ATP Molar ratio in three species studied is 4-10:1. Studies of the turnover of adenine nucleotides in cholinergic sympathetic vesicles have shown that ATP and Ach are depleted to the same extent (about 50%) during nerve stimulation (Zimmerman et al 1979). It is also

known that ATP is stored and released together with catecholamines from adrenal chromaffin cells (Burnstock 1985). Storage of ATP together with NA in adrenergic nerves was recognized in the early literature. The first indication that ATP might be released from adrenergic neurons was the demonstration that stimulation of periarterial adrenergic nerves led to release of tritium from taenia coli preincubated in [^{3}H] adenosine; both the release of tritium and NA were blocked by guanethidine (Su et al 1971).

The coexisting substances NA and ATP, act as synergic neurotransmitters via post-junctional receptors, as well as exerting modulatory effects on each other via pre- and post junctional mechanism (Burnstock 1985). But the real functional significance of these type of coexistence needs more exploration.

In recent years, co-existence of neuropeptide Y (NPY) and several other peptides has been observed (McDonald 1988). Accordingly, NPY displays a wide variety of functional activities depending on its location and co-existence with other substances, especially catecholamines. NPY is extensively known to be distributed throughout the cardiovascular system in many species (Edvinsson et al 1983, Schon et al 1985, Allen et al 1986).

VI. Knee joint blood vessels.

In the literature, there is little information about the physiology of blood flow to the joints. It seems that a lack of a suitable animal preparation for study of the properties of articular blood vessels is one of the main reasons, although, some attempts have been made in the late 1940's and 1950's to estimate joint blood flow indirectly in man by measurement of intra-articular temperature (Horvath and Hollander 1949), and by the application of the plethysmograph to a knee segment (Bonney,et al 1952), and to show the effect of heating, cooling, and drugs such as adrenaline and noradrenaline directly on articular blood vessels in the dog (Cobbold and Lewis 1956a,b,c).

The behaviour of the joint circulation when the external temperature of the joint is increased was studied by Horvath and Hollander (1949), who showed that increase and decrease in temperature of the joint cause vasoconstriction and vasodilation respectively. This was not supported by the result of Hunter and Whillans (1951), using the same technique (application of a hot and cold packs to the joint in man), since they observed that the joint temperature fell when their subject was exposed to cold, and this they attributed to a reflex superficial vasodilatation resulting in a short period of excessive heat loss. Bonney and his colleagues found that cooling the segment enclosed in the plethysmograph resulted in a fall in blood flow and heating did vice-versa. It is important to mention that the use of the knee segment in the plethysmograph device as a method for articular blood flow

measurement is open to an obvious disadvantage in that blood flow to considerable amount of other tissues were also measured. This disadvantage was overcome out by direct measurement of blood flow to knee joint in dog (Cobbold and Lewis 1956a,b). They concluded that 1) the rise in temperature of the joint caused an increase in blood flow, and fall in temperature decrease in flow, 2) adrenaline and NA administered intra-arterially caused a constriction of joint blood vessels, 3) NA produced consistently greater responses than adrenaline, 4) intra-arterial injection of acetylcholine cause dilation of the joint blood vessels. In summary they stated that at least in regard to response to temperature changes and to intra-arterial adrenaline and NA, the joint blood vessels behave like those of skin.

The innervation of joint blood vessels is another important issue to be considered. Although the somatic innervation of joint has been reviewed by Gardner in 1950, little is still known about the autonomic control of articular blood vessels. It has been assumed that, since the joint structures are provided with sympathetic innervation similar to that of blood vessels of other deep structures, the control of the joint vessels follows a similar pattern. Cobbold and Lewis (1956b) observed that stimulation of the sympathetic chain resulted in a reduction of blood flow to the dog knee joint.

In a recent study on the knee joint of cat, electrical stimulation of the posterior articular nerve caused an initial vasoconstriction of the blood vessels

followed by a long lasting dilatation (Ferrell and Cant 1987).

However, the nature of the neurotransmitters and types of receptor(s) which mediate these vasoconstrictor and vasodilator resposes were not investigated in either of these studies.

****

****

****

*****

***

*

CHAPTER TWO

GENERAL
MATERIAL AND METHOD
IN THE RABBIT

A: Materials

I. Instruments:

1. Dissecting microscope M650 WILD (Switzerland)
2. Dissecting table, Palmmer (England)
3. Ventilator pump, Palmmer (England)
4. Pressure transducer, Elcomatic EM750 (Scotland)
5. Clock, PYE (England)
6. Spectrophotometer LKB-Ultrospec II (England)
7. Balance, Metler AE50 European Instrument, Oxford
8. Water bath, Grant Instrument LTD (England)
9. Slow infusion apparatus, SRI (United Kingdom)
10. Pump, Watson-Marlow (England)
11. Cordless Cautery, Warecrest. C28. (England)
12. Whirlimixer (England)
13. Laser blood flow - MBF2, Moor Instrument (England)
14. Laser blood flow - MBF3, Moor Instrument (England)
15. Thermalert Model TH-6D, Harvard (U.S.A.)
16. Neurolog System; Pressure ampilifier, Delay-Width, Spike trigger, Pressure buffer, Digital-Width, Pulse buffer, (England)
17. Multitrace polygraph, Lectromed (England)
18. Rate meter, Lectromed (England)
19. Oscilloscope 5103N (England)
20. Centrifuge, Super Medium - MSE (England)

21.Advance stimulator, Harvard (U.S.A.)

22.Shaker, Heidolph (W. Germany)

23.Minipuls 2, Gilson (France)

24.Significat Scat 01, Digitimer LTd. (England)

25.Nescofilm Bando-Chemical IND, LTD,(Japan)

26.Polythene cannulas in sizes of 2FG, 3FG, 4FG, 5FG, Portex LTD, (England)

27.Syringe, 1,2,5,10,20ml, Plastipak (England)

II. Drugs:

Drugs which have been used for either the in-vitro or in-vivo preparation were;

1.Pentobarbitone sodium, (Sagatal; May & Baker LTD Dagenham).

2.[arg$_8$]-Vasopressin acetate (Sigma),

3.Adrenaline hydrochloride (Sigma)

4.Noradrenaline hydrochloride (Sigma)

5.Phenylephrine hydrochloride (Sigma)

6.Angiotensin II, Acetate salt (Sigma)

7.Propanolol hydrochloride (Sigma)

8.Phenoxybenzamine hydrochloride (Smith, Kline & French)

9.Isoprenaline sulphate (Aldrich)

10.UK-14304 bitartarate (5-bromo-6[2-imidazolin-2-Ylamino]-quinoxaline) (Pfizer)

11.Prazosin hydrochloride (Pfizer)

12.guanithidine sulfate (Ciba)

13. α , β methyleneadenosine 5'-triphosphate, lithium salt (Sigma)

14.Tetrotodoxin (Sigma)

15.Evans blue (Sigma)

16.Kaolin, hydrated aliminum silicate (Sigma)

17.Adenosine (Sigma)

18.Adenosine 5'-triphosphate, lithium salt (Sigma)

19.5-Hydroxy-Tryptamine hydrochloride, 5HT (Sigma)

20.Deoxycholic acid, sodium salt (Sigma)

21.Acetylcholine hydrochloride (Sigma)

22.3-methylxanthine (Sigma)

23.Atropine sulfate (Sigma)

24.Rauwalscine hydrochloride (Roth)

25.Cimetidine (Aldrich)

26.Histamine dihydrochloride (Sigma)

27.Diazepam BP with benzyl alcohol and sodium benzoate (Roche)

28.Hypnorm (Janssen Pharmaceutical Ltd)

29.Heparin sodium (Evans)

30.^{57}Cobalt radioisotope (Dupont)

31.^{113}Tin radioisotope (Dupont)

32.^{153}Gadolinium radioisotope(Dupont)

33.^{46}Scandium radioisotope(Dupont)

34.Schlesinger medium, Gelatin-KI-barrium sulfate mass (Schlesinger 1957)

35.Sodium sulfate anhydrous (May & Baker LTD, England)

36.Acetone, (May & Baker LTD, England)

37.Capsaicin, Fluka AG, CH-9470 Buchs (Switzerland)

38.Substance P antagonist (D-Pro4,D-Trp 7,9,10)-SP(4-11), (Peninsula Laboratories,Inc)

39.Reserpine (Sigma)

40.Normal saline

41.Lockes solution; Its composition per litre is (mM): NaCl, 115; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4 . 7H_2O$, 1.2; $NaHCO_3$, 24.1; KH_2PO_4, 1.2; and glucose, 5.6. $CaCl_2$ was added after oxygenating the solution with a 95% O_2 and CO_2 mixture.

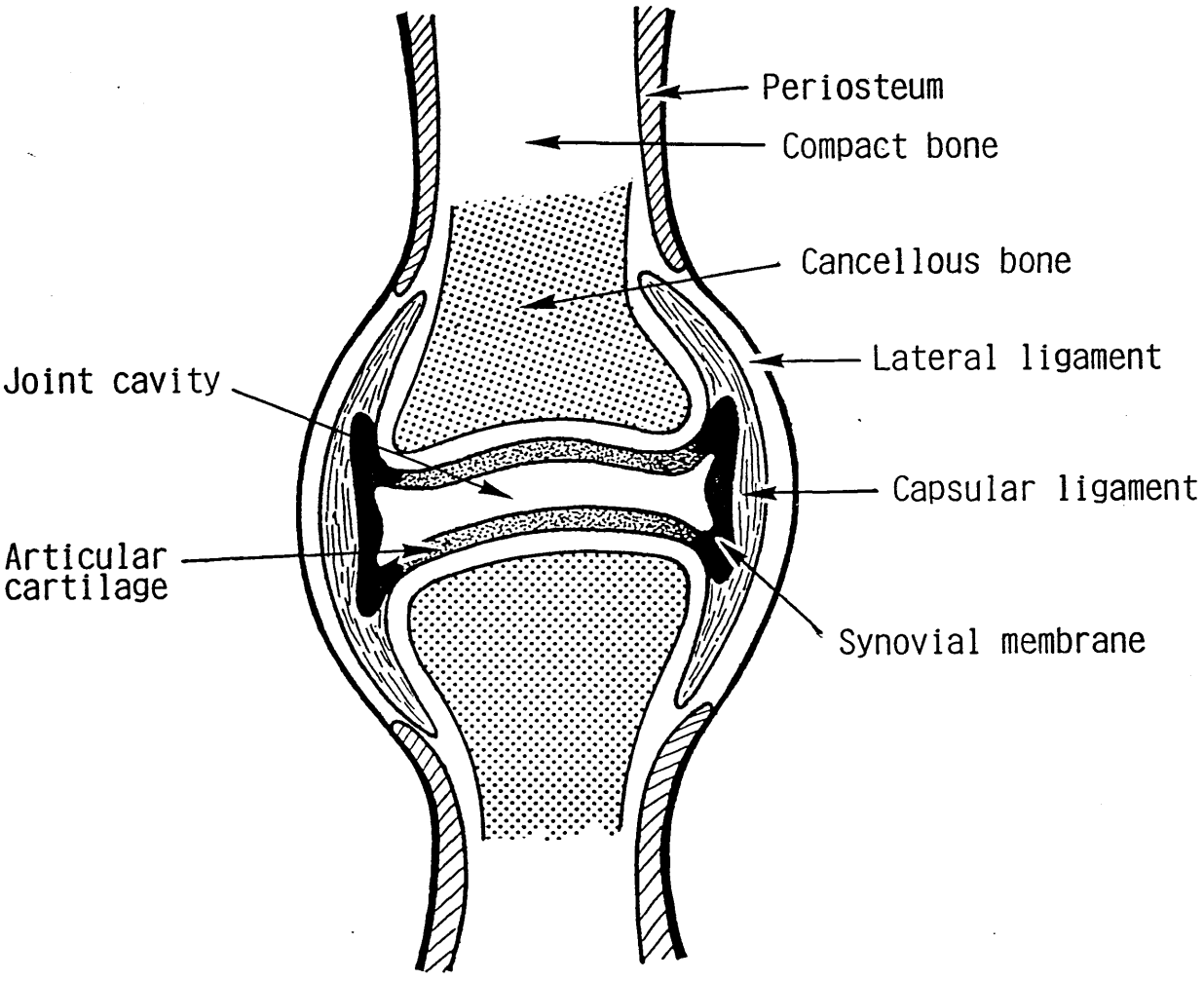
B: Methods

I.Surgical anatomy

It is conventionally supposed that each articulation is enclosed in a capsule formed by a sheet of strong connective tissue extending from one bone to other and completely enclosing a cavity containing the articular surfaces. Each of these surfaces is covered by a very thin layer of cartilage and the cavity contains a small amount of lubricating liquid, the synovial fluid secreted by the lining of the capsule, the synovium (Figure 2.1).

The articulation in the knee forms a hinge-joint, though movement is not rigidly confined to one plane. The structure is more complex than of other joints (e.g. hip joint). The capsule is attached to the femur around the edges of the condyles and the patellar surface and similarly to the tibia round the edges of its condyles. Outside the capsule, stout medial and lateral bands, the tibial and fibular collateral ligaments, hold the two bones together. The capsule is also attached to four small bones which have articular surfaces taking part in the formation of the joint, namely the patella and three other sesamoid bones embedded in the two heads of the gastrocnemius and in the popliteus muscles respectively. The patellar ligament forms part of the capsule and the joint is transversed by the tendons of origin of the

Fig. 2.1. **The structural view of a diarthrodial joint.** Showing **synovial cavity and membrane.**



A Diarthroidal Joint

popliteus and of the extensor digitorum longus.

Within the joint the femur and the tibia are held together by an anterior cruciate ligament and posterior cruciate ligament, both attached to the femur in the intercondyloid fossa. Curved cushions of cartilage, the medial and lateral menisci, are inserted between the apposed condyles and are held in place by additional ligaments. The medial meniscus is attached to the tibia only but the lateral meniscus is connected to both tibia and femur , the ligament to the femur lying behind the posterior cruciate ligament.

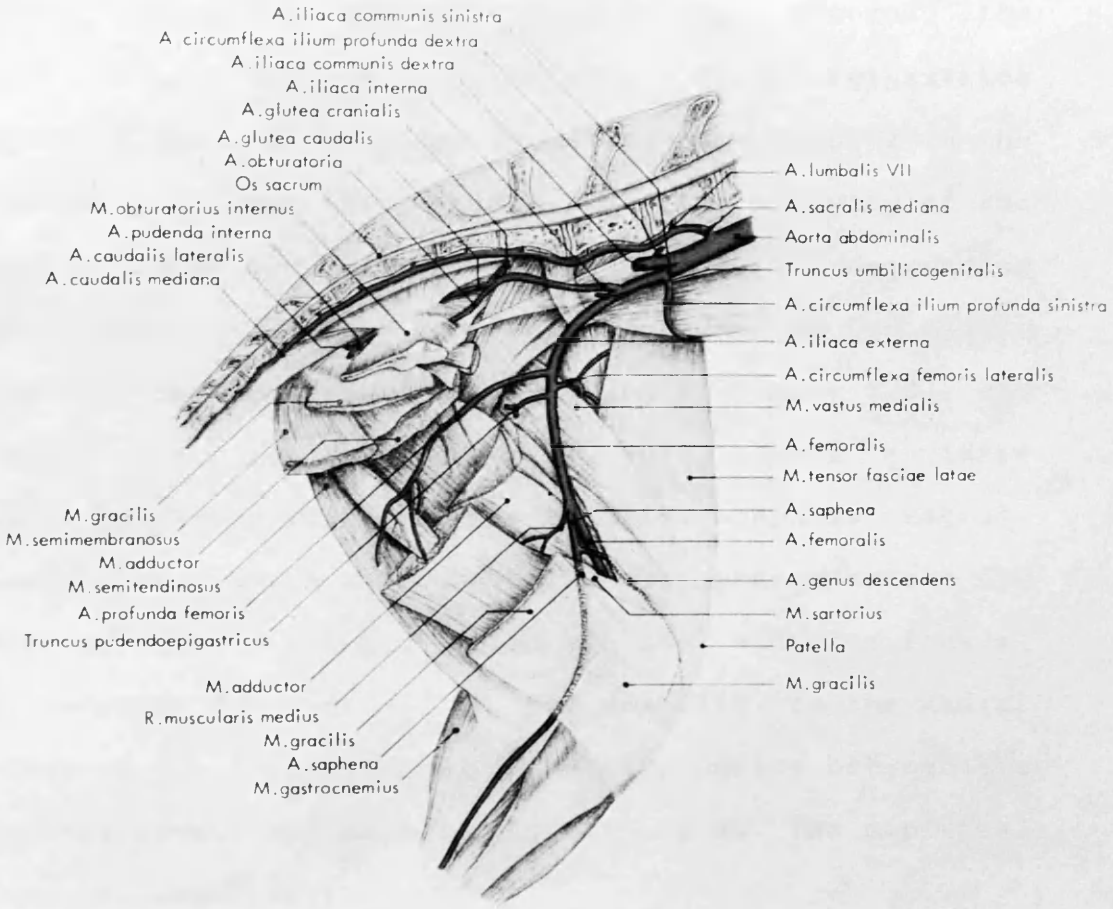
The capsules at the knee joint should be exposed by removing the muscular attachments about them as thoroughly as is practicable.

Blood vessels and nerves to knee joint

a:Arteries

The femoral artery transverses the medial surface of the thigh, begining at the dorsal side of the inguinal ligament, where it continues from the external iliac artery (Figure 2.2). Immediately distal to the inguinal ligament it gives off posteriorly the deep artery of the thigh (artery profunda femoris). The latter passes to the dorsal side of the pectineus and adductor brevis muscles and distributed to the posterior proximal portion of the limb, chiefly to the adductors longus and magnus (Figure 2.3). A second branch, the lateral circumflex artery (a.

Fig. 2.2. The arteries of the pelvis and of the thigh. The medial aspect of rabbit hind limb(Barone et al, atlas of rabbit anatomy).



ARTERIAE PELVIS ET FEMORIS (FACIES MEDIALIS).

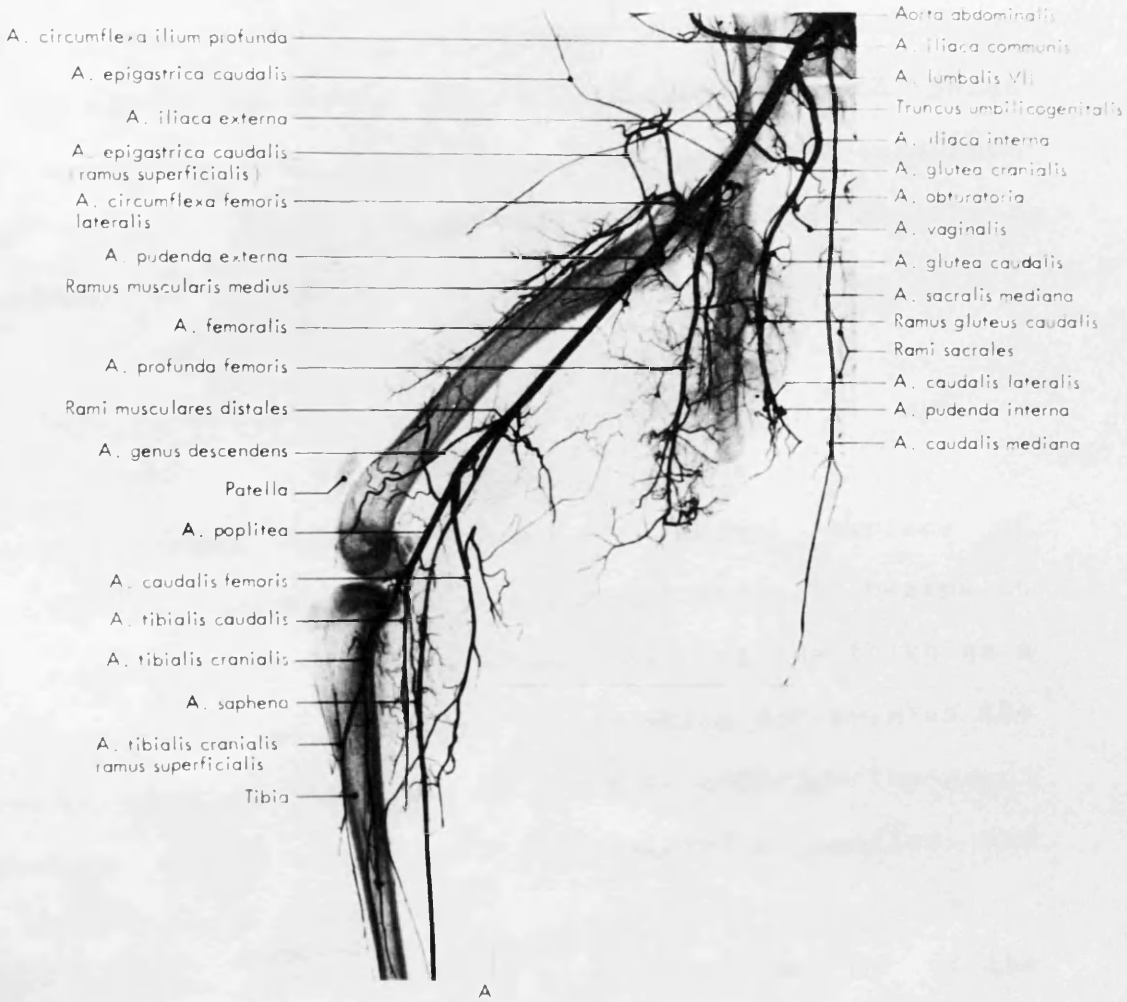
Artères du bassin et de la cuisse (face médiale).

Arteries of the pelvis and of the thigh (medial aspect).

Circumflexa femoris lateralis), is given off from the anterolateral wall. It passes between the second head of the rectus femoris and vastus lateralis, on the one hand, and the two portions of the vastus intermedius, on the other hand. It supplies various parts of the quadriceps femoris group. A third branch of the femoral, the superficial epigastric artery (a. epigastrica superficialis), given off medially, and passing to the abdominal wall, has been divided. At the begining of the distal third of the thigh, a small branch, the descending genus artery (a. genu suprema), passes over the medial condyle of the femur to the knee joint (Figure 2.3), and at about the point of origin of this vessel a large branch, the great saphenous artery (a. saphena magna), arises from the posterior wall. It passes across the medial surface of the distal end of the adductor longus, and through the tendon of the gracilis, to the medial surface of the leg. The femoral artery passes between the adductors longus and magnus , continuing as the popliteal artery (a. poplitea).

The popliteal artery, the continuation of the femoral, passes between the medial head of the gastrocnemius on the one hand and the lateral head of the plantaris on the other, reaching the anterior surface of the popliteus, and afterwards the anterior surfaces of the tibia and fibula by passing between their proximal ends. It distributes branches to the muscles and joint about the knee-joint, including a branch to the distal portion of the vastus lateralis, which is given off near the same

Fig. 2.3. The radiograph of arteries of the pelvis and hind limb of rabbit (Barone et al, atlas of rabbit anatomy).


A. circumflexa ilium profunda
A. epigastrica caudalis
A. iliaca externa
A. epigastrica caudalis (ramus superficialis)
A. circumflexa femoris lateralis
A. pudenda externa
Ramus muscularis medius
A. femoralis
A. profunda femoris
Rami musculares distales
A. genus descendens
Patella
A. poplitea
A. caudalis femoris
A. tibialis caudalis
A. tibialis cranialis
A. saphena
A. tibialis cranialis ramus superficialis
Tibia
Aorta abdominalis
A. iliaca communis
A. lumbalis VII
Truncus umbilicogenitalis
A. iliaca interna
A. glutea cranialis
A. obturatoria
A. vaginalis
A. glutea caudalis
A. sacralis mediana
Ramus gluteus caudalis
Rami sacrales
A. caudalis lateralis
A. pudenda interna
A. caudalis mediana

A

point as the small saphenous artery. It then continues as the anterior tibial artery (a. tibialis cranialis). The other branch which runs posteriorly is called as posterior tibial artery (a. tibialis caudalis).

As described by Liew and Dick (1981), the sources of origin of the arterial blood supply to the joint are multiple. The vessels from the popliteal artery and other vessels from the bones and muscle adjacent to joint anastomose freely to form a complex network around knee joint between the capsule and the synovium at their attachment to epiphysial line.

b:Veins

The femoral vein transverses the medial surface of the thigh in company with the femoral artery. It begins at the proximal end of the lower third of the thigh as a continuation of the popliteal vein, which accompanies the corresponding artery. Its tributaries comprise the great saphenous, superficial epigastric, lateral circumflex, and the deep vein of the thigh.

The great saphenous vein, a large tributary of the femoral, accompanies the corresponding artery and the great saphenous nerve. It is a continuation of the posterior tibial vein from the plantar surface of the foot.

The popliteal vein, the root of the femoral, accompanies the corresponding artery in the popliteal fossa. It receives the small saphenous vein from the

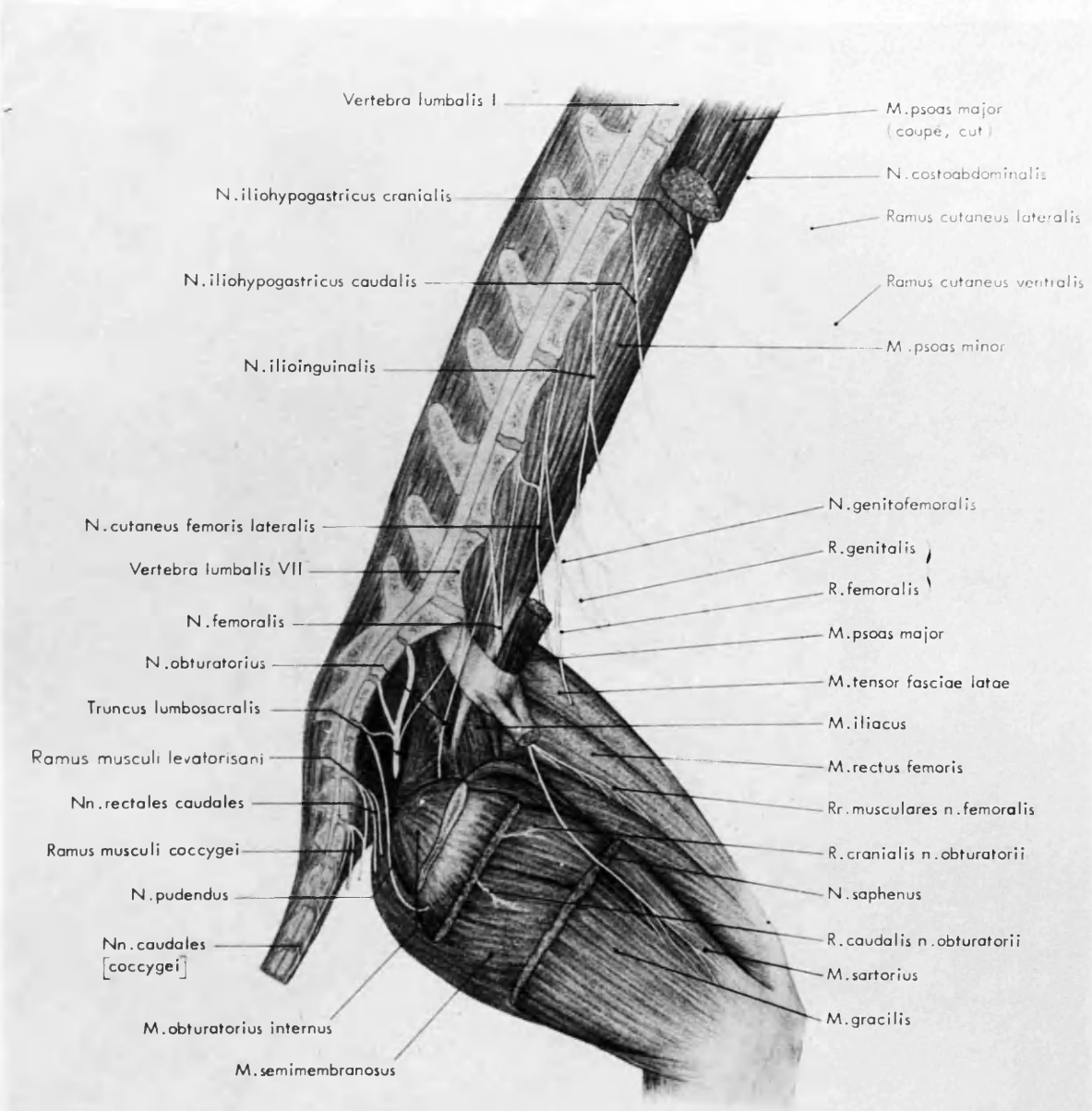
posterior margin of the lateral head of the gastrocnemius, where this vein has been formed by tributaries accompanying the distal branches of the small saphenous artery.

c.Nerves

The femoral nerve (n. femoralis) arises from the lumbo-sacral plexus, chiefly from the fifth and sixth lumbar nerves (Figure 2.4). Its position between the psoas major and iliacus muscles has been shown in figure 2.4. Immediately beyond the inguinal ligament it divides into two portions, one of which is distributed to the muscles of the anterior side of the thigh, while the other, the great saphenous nerve (n. saphenus major), passes to the medial surface of the thigh and leg in company first with the femoral artery and afterwards with the great saphenous artery.

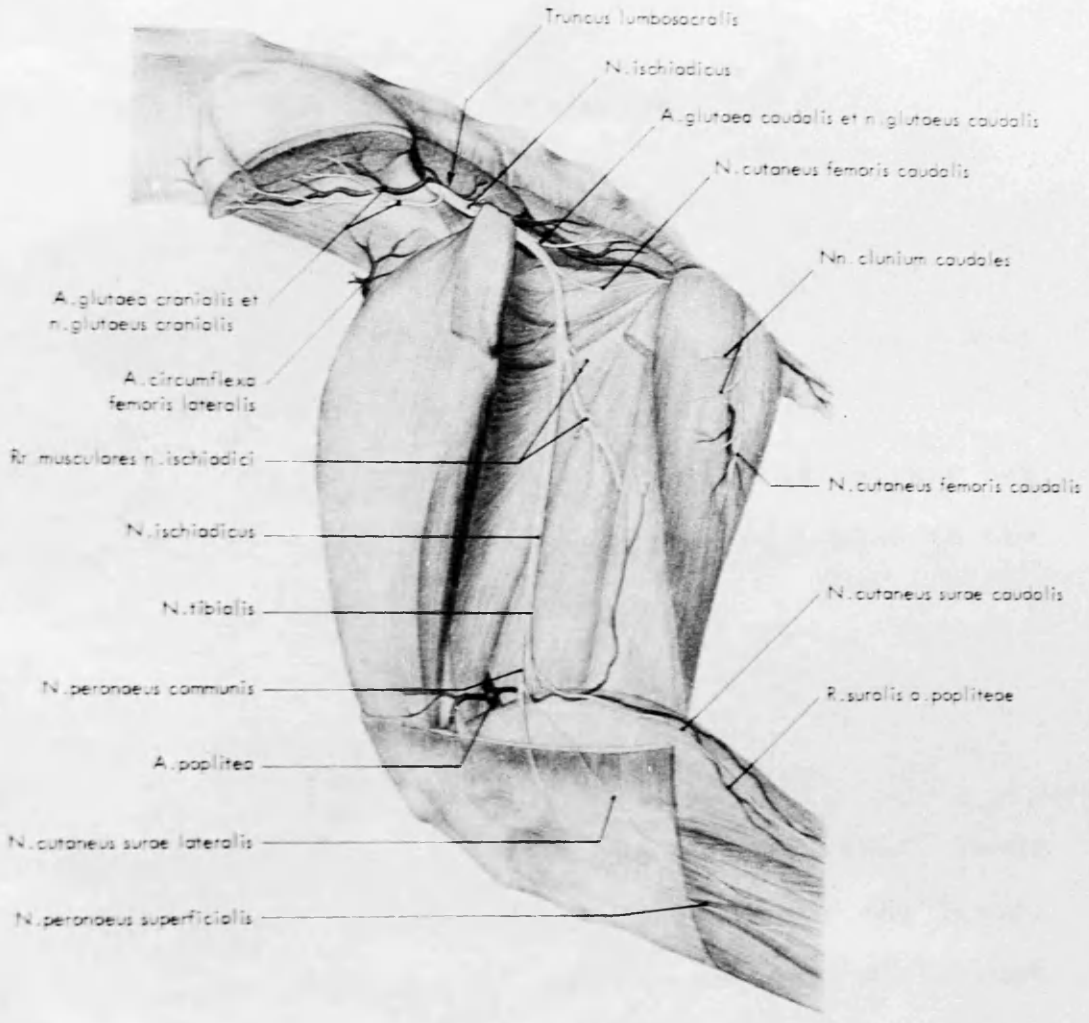
The sciatic nerve (n. ischiadicus), formed chiefly from the seventh lumbar and first sacral nerves, appears laterally in the greater sciatic notch. It passes backward beneath the pisiformis muscle, and then turns and extends distal through the thigh, where it lies on the lateral surfaces of the adductors magnus and longus (Figure 2.5). It distributes branches to the posterior musculature of the thigh. In the proximal portion of the thigh it divides into two chief branches, which are closely associated as far as the knee. The anterior branch is the peroneal nerve (n. peronaues), the posterior branch the tibial nerve (n.

Fig. 2.4. The saphenous nerve and its anatomical position. The medial aspect of rabbit hind limb (Barone et al, atlas of rabbit anatomy).



PLEXUS LUMBOSACRALIS.
Plexus lombo-sacré.
Lombosacral plexus.

Fig. 2.5. The sciatic nerve and its branches. The lateral aspect of rabbit hind limb (Barone et al, Atlas of rabbit anatomy).



NERVUS ISCHIADICUS.

Nerf sciatique.

Sciatic nerve.

tibialis). The lesser saphenous nerve is a small branch given off from the tibial above the knee joint. The posterior articular nerve (PAN) which supplies the dorsal aspect of the knee joint branches off from the tibial nerve just below the knee joint.

II. Animals and tissue preparation

a. In-vitro experiments

1. Animals:

154 adult albino New Zealand rabbits of either sex weighing between 1.7-4.5 Kg, were killed by a blow to the skull and exanguinated immediately.

2. Tissue preparation:

Immediately after killing the rabbit, the lower limbs were cut off from proximal position of the femur. One limb was transferred to the dissecting microscope while the other was kept in cool place for further surgical procedures. The posterior aspect of the knee joint was exposed, the popliteal artery was gently separated from the popliteal vein and one 10 cm polythene cannula (size 3 or 4 FG) was used for cannulation of the artery. All muscular branches of popliteal artery were ligated but the articular branches were left unligated, as

shown in figure 2.6. The tissue was perfused with warmed and oxygenated Locke solution by means of a peristaltic pump (Gilson minipuls). The extra part of femur above the cannulation and the lower part of the limb midway through the tibia were removed by sawing the bones.

The isolated knee joint preparation was then transferred to a thermostatically controlled bath (37 ± 1 °C) containing oxygenated Locke solution (Figure 2.7). pO_2, pCO_2, and pH of the locke's solution were measured and found to be 200-300 mmHg, 30-34 mmHg, and 7.42-7.46 respectively. As the effect of O_2 level was studied by some investigators, they showed that although this level of PO_2 caused constriction in vascular smooth muscle but was not significant (Maclennan et al 1988).

3. Recording and Measurements:

At the start of the experiment the pump was set to provide a perfusion rate of 0.5-0.9 ml/min which resulted in a perfusion pressure of about 40mmHg as measured by a pressure transducer connected "downstream" from the pump (Figure 2.8).

Changes in perfusion pressure thereafter provided an indirect measure of articular blood vessel calibre. Since, according to the equation:

$$\Delta P = F \times R$$

Fig. 2.6. **Diagrammatic representation of the arterial supply of the dorsal aspect of the rabbit knee joint capsule.**

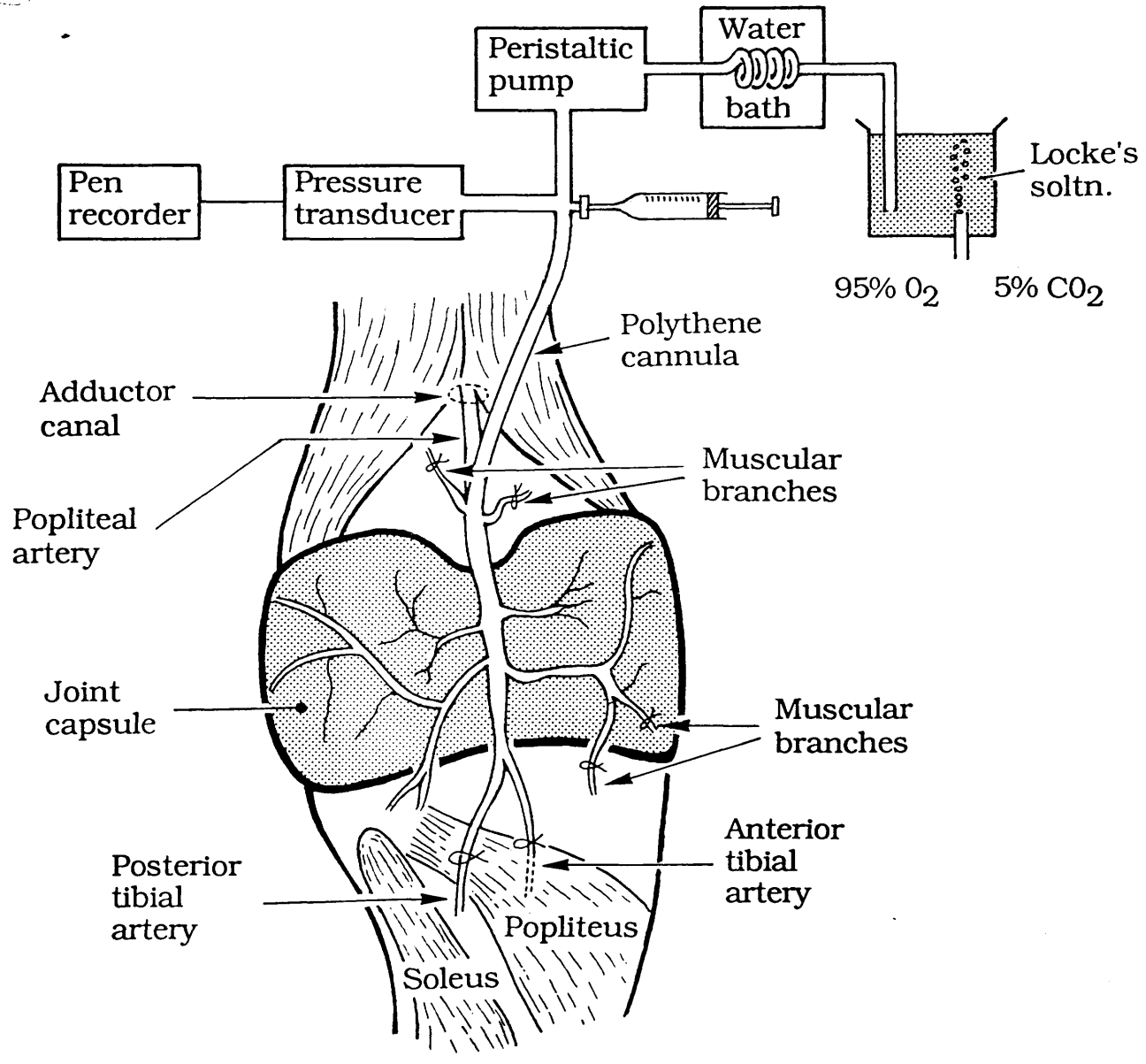

Water bath
Peristaltic pump
Pen recorder
Pressure transducer
Locke's soltn.
95% O_2
5% CO_2
Polythene cannula
Adductor canal
Muscular branches
Popliteal artery
Joint capsule
Muscular branches
Anterior tibial artery
Posterior tibial artery
Popliteus
Soleus

Fig. 2.7. Diagrammatic representation of the organ bath for in-vitro experiments.

1. Inlet tube for O_2 (95%), and CO_2 (5%) gas mixture.
2. Inlet tube for Locke solution
3. Outlet tube for exchange of fresh Locke solution.
4,5. Inlet and outlet tubes for circulation of warm water under the organ bath to keep appropriate temperature constant.

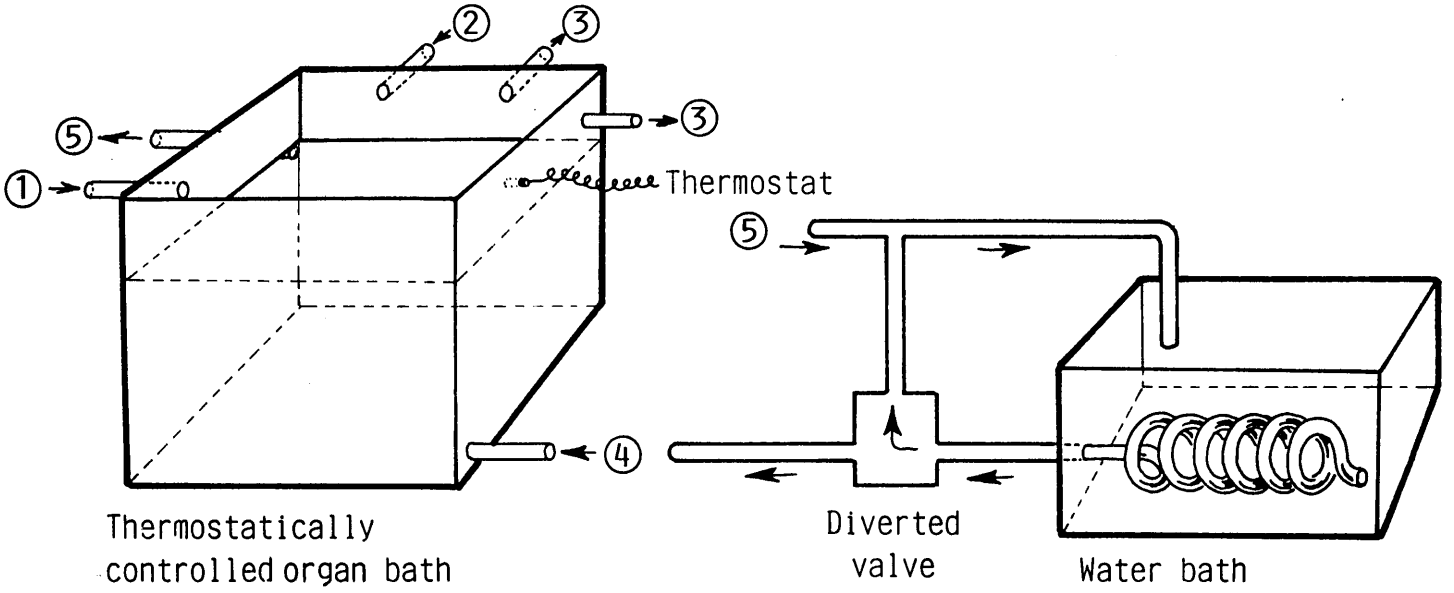
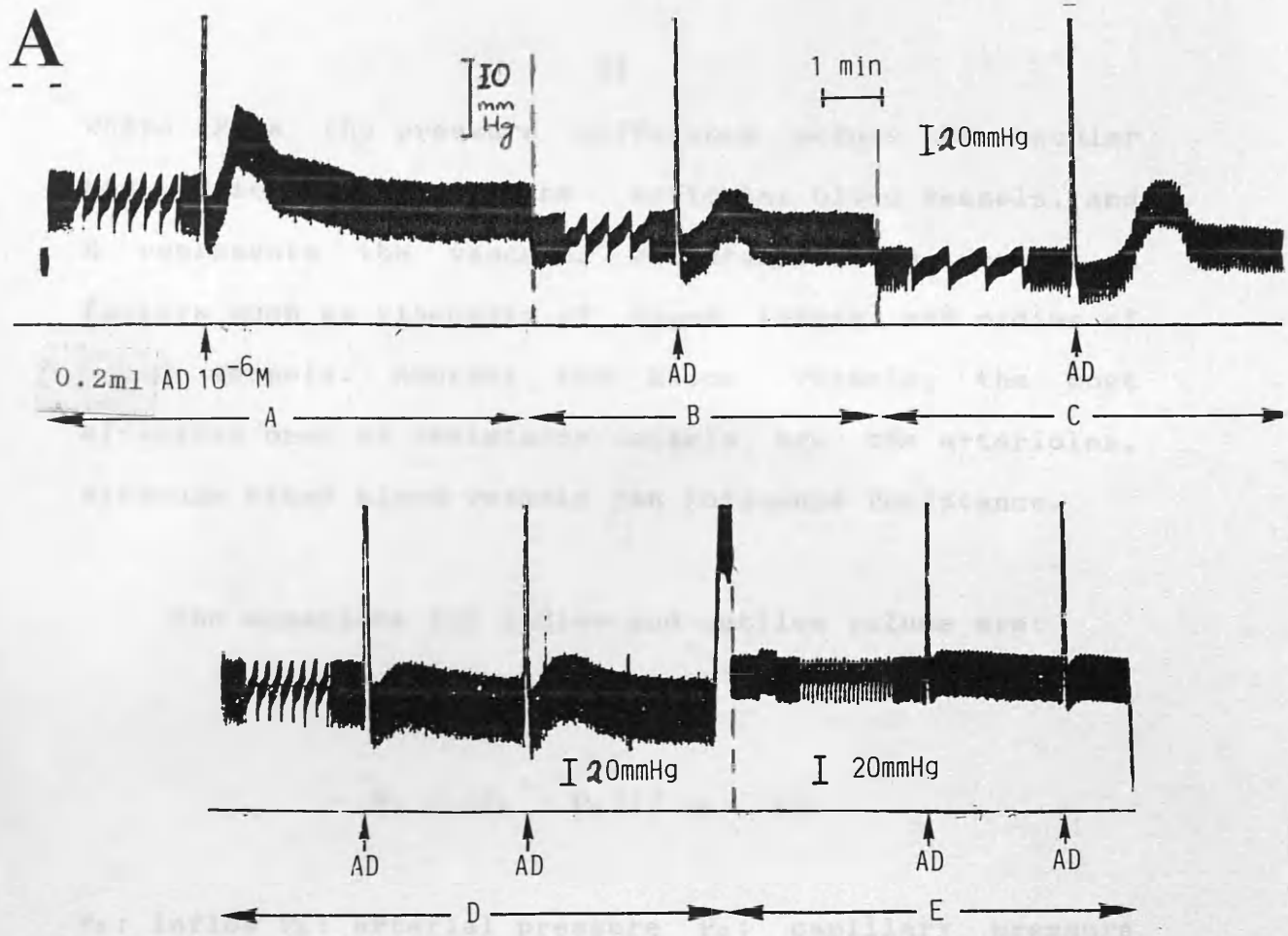
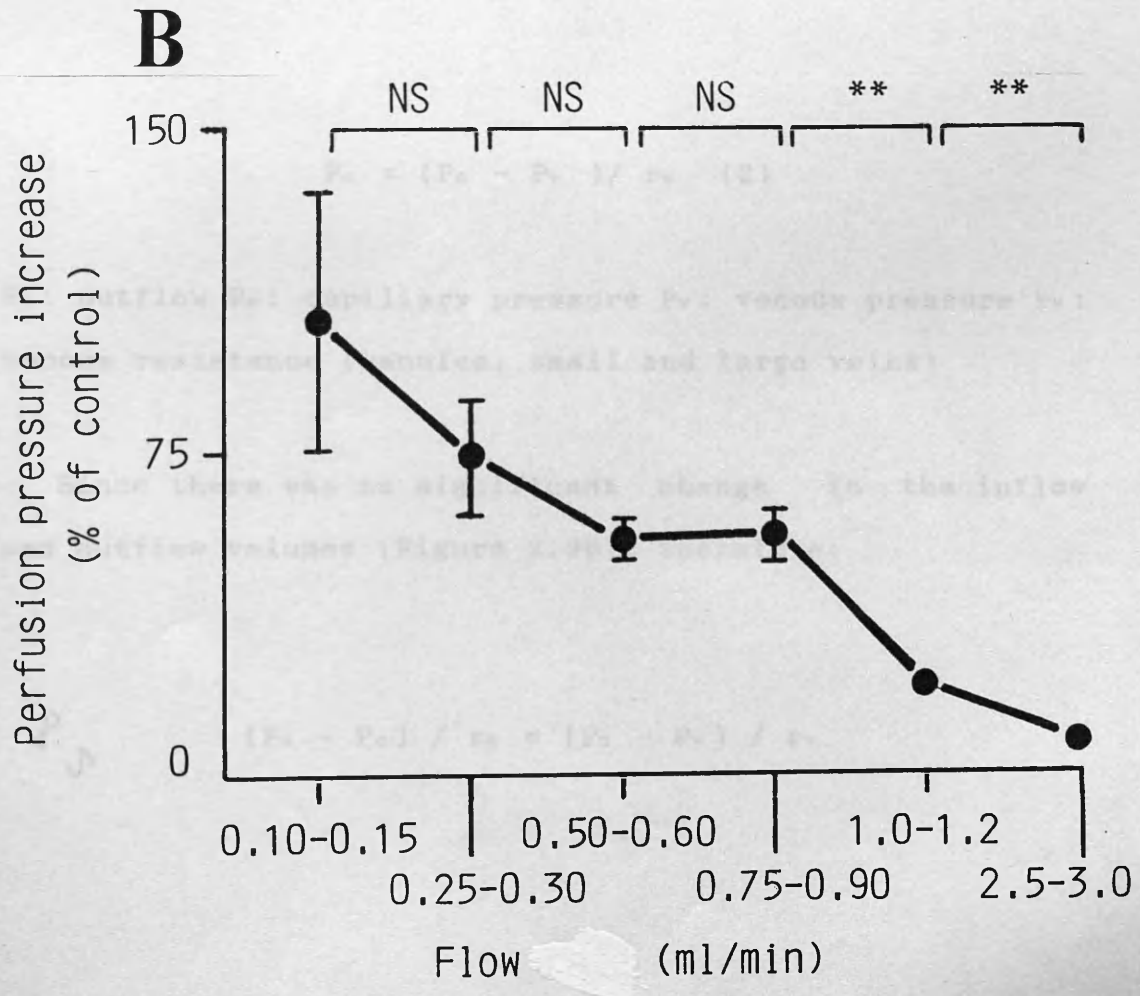

②
③
⑤
③
①
Thermostat
⑤
④
Thermostatically
controlled organ bath
Diverted
valve
Water bath

Fig. 2.8. A:Traces show the effect of adrenaline (10^{-6}M) injection at different perfusion rates: 0.25-0.3 (A), 0.5-0.6 (B), 0.75-0.9 (C), 1.0-1.2 (D), 2.5-3ml/min (E).

B: The response curve to injection of adrenaline (10^{-6}M) at different perfusion rates. It seems that the effect at 0.5-0.9ml/min is optimal.


A
10 mm Hg
1 min
20mmHg
0.2ml AD 10⁻⁶M
AD
AD
A
B
C
20mmHg
20mmHg
AD
AD
AD
AD
D
E


B
NS
NS
NS
**
**
150
75
0
Perfusion pressure increase
(% of control)
0.10-0.15
0.25-0.30
0.50-0.60
0.75-0.90
1.0-1.2
2.5-3.0
Flow (ml/min)

where P is the pressure difference across the vascular bed . F is flow through the articular blood vessels, and R represents the vascular resistance which depends on factors such as viscosity of blood, length, and radius of blood vessels. Amongst the blood vessels, the most effective ones as resistance vessels are the arterioles, although other blood vessels can influence resistance.

The equations for inflow and outflow volume are:

$$F_i = (P_a - P_c)/ r_a \quad (1)$$

F_i: inflow P_a: arterial pressure P_c: capillary pressure r_a: arteriolar resistance

$$F_o = (P_c - P_v)/ r_v \quad (2)$$

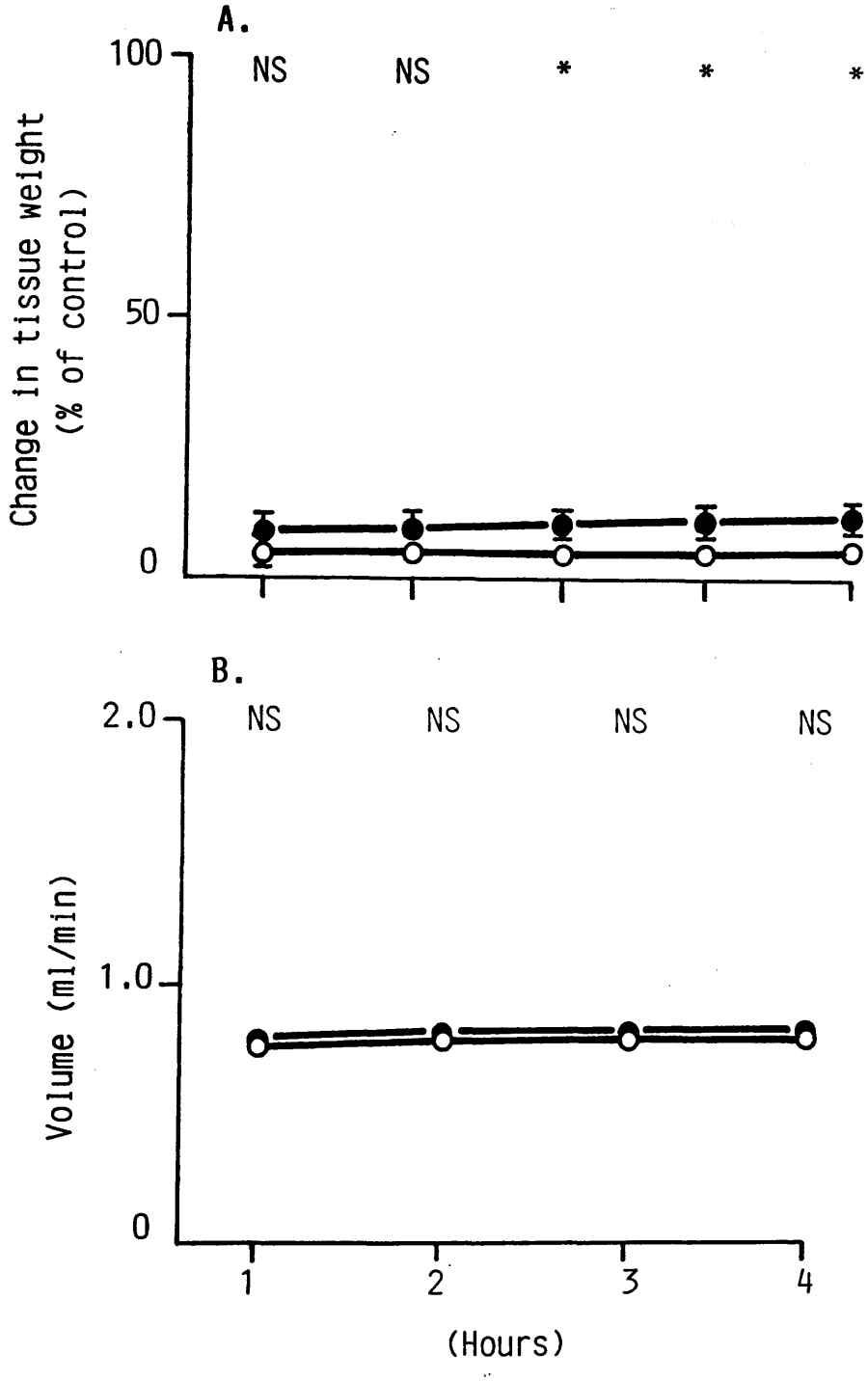
F_o: outflow P_c: capillary pressure P_v: venous pressure r_v: venous resistance (venules, small and large veins)

Since there was no significant change in the inflow and outflow volumes (Figure 2.9B), therefore:

$$(P_a - P_c) / r_a = (P_c - P_v) / r_v$$

Fig. 2.9. A: Changes in weight of the knee joint after perfusing with Locke solution for 5 hours (●) and by placing the knee joint in the bath for same period of time but without perfusion(O). n=6, * $P<0.05$

B: Input volume of Locke solution perfused through articular blood vessels (●) compared with output volume (O) during 4 hours of perfusion.


A.
100
50
0
NS
NS
*
*
*
Change in tissue weight
(% of control)
B.
2.0
1.0
0
NS
NS
NS
NS
Volume (ml/min)
1
2
3
4
(Hours)

Also, as there is no tonic sympathetic input nor circulating vasoconstrictor hormones, the preparation is maximally dilated. Since then, the tissue weight is little changed (Figure 2.9A), P_c is unlikely to change significantly, and changes in r_v related to r_a is very small, therefore if the flow was kept constant, any changes in pressure is due to changes in vascular resistance, mainly in the arterioles. So, changes in perfusion pressure thereafter provided an indirect measurment of articular blood vessels calibre.

Control injections of Locke solution were administered periodically and were found to produce little change in pressure, apart from a transient rise during the injection phase. After injection or perfusion of different drugs, peak response was compared to the control (pre injection) value and expressed as percentage change from control (or baseline). Where drugs were injected, these were administered by 0.2ml bolus over two seconds. The concentrations shown in the figures refer to that in the syringe and can be converted to the mass of drug injected by multiplication of that concentration by $2X10^{-4}$.

b. In-vivo experiments:

1. Initial surgical procedures in rabbit

(i): Animals

54 adult white New Zealand rabbits were used, their weights ranging from 1.8Kg to 4.6Kg.

(ii): Anaesthesia

Anaesthesia was initially induced by intra-muscular injection of hypnorm (0.1mg/Kg) and an intra-peritoneal injection of diazepam (0.5mg/Kg). No surgical procedure was performed until reflex withdrawal of the limb on pinching the paw had been abolished. Thereafter, anaesthesia was maintained using a gaseous mixture of oxygen (O_2) and nitrous oxide (N_2O) and halothane (1-2%) which was delivered via a tracheal cannula.

(iii): Tracheotomy

The fur on the neck was shaved with fur clippers and a skin incision made from the hyoid bone to the suprasternal notch. The skin was retracted and the pretracheal muscles were separated by blunt dissection to expose the trachea. Any overlying connective tissues were cleared by blunt dissection, and a thread was looped around the trachea. The trachea was then then lifted by this thread and an incision was made between two trachea rings. A glass trachea cannula of appropriate size was then inserted into the trachea and tied in position with the thread.

(iv): Carotid artery cannulation

In all experiments, either right or left or both common carotid arteries were cannulated to permit

monitoring of arterial blood pressure, or withdrawal arterial blood samples and /or insertion of a cannula into the left ventricle. The carotid was first freed from surrounding connective tissues and the vagi, then the distal portion of common carotid was ligated with thread, another loose tie was placed on the carotid proximal to the ligated tie. By pulling the loose tie proximal blood flow stopped at 2-3cm below the ligated tie, then an small incision was made between two ties and a heparinized cannula was then secured in position. The ligatures were firmly secured to stop movement of the cannula.

The skin incision in the neck was then closed over with Michel clips.

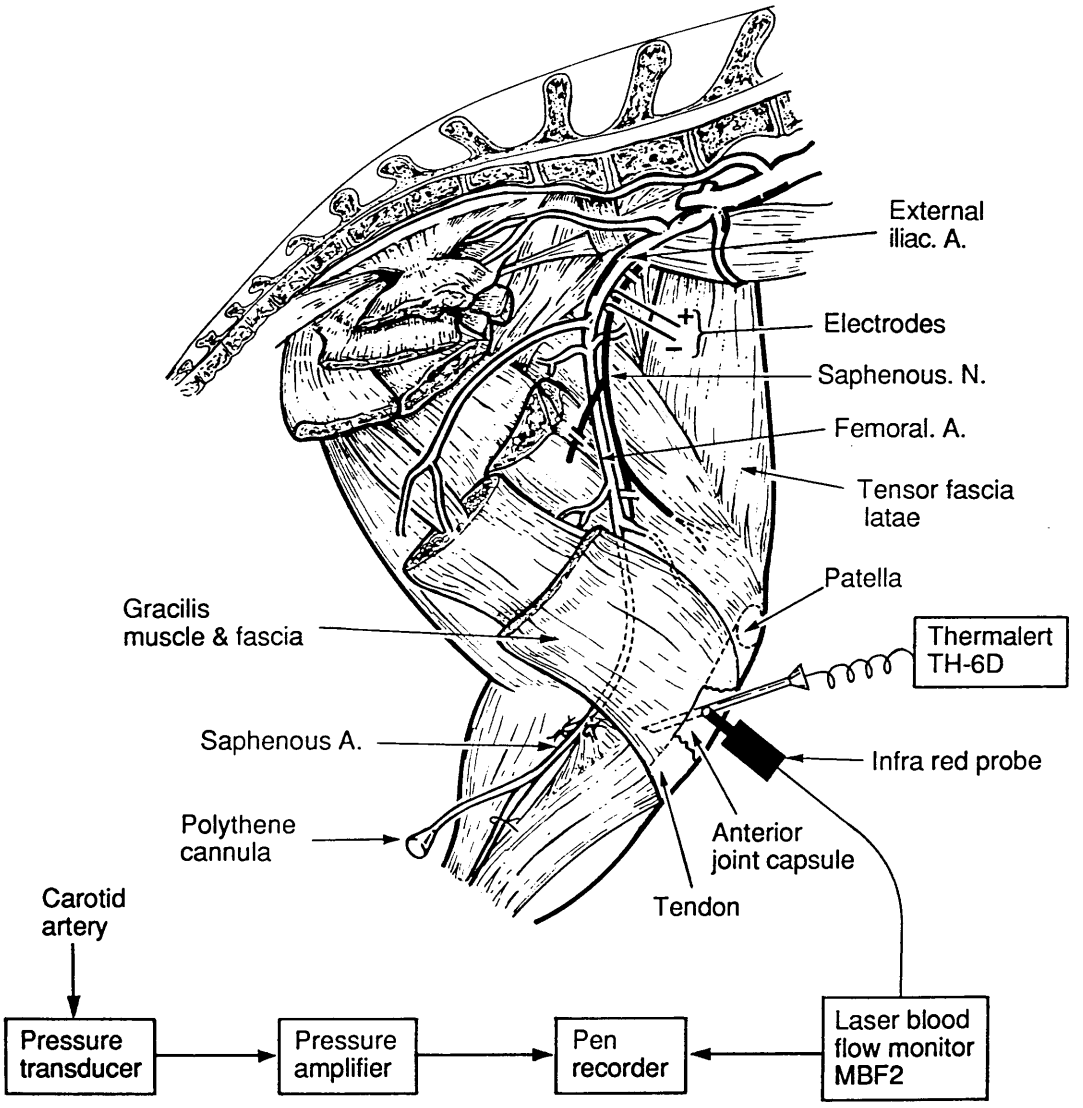
(v): Saphenous artery cannulation

Where intra-arterial injection of drugs close to knee joint was necessary, a 25G polythene heparinized cannula was inserted into the saphenous artery in a retrograde direction and advanced rostrally until the tip of the cannula was just below the branches supplying the knee joint (Figure 2.10). Non-articular branches were ligated.

(vi): Temperature regulation

The animal's temperature was maintained at around 37 °C by means of a heated operating table during the surgical and experimental procedures. Body temperature was

Fig. 2.10. Diagram of the experimental arrangement. View of the medial aspect of the rabbit knee.


External iliac. A.
Electrodes
Saphenous. N.
Femoral. A.
Tensor fascia latae
Patella
Gracilis muscle & fascia
Thermalert TH-6D
Saphenous A.
Infra red probe
Polythene cannula
Anterior joint capsule
Tendon
Carotid artery
Pressure transducer
Pressure amplifier
Pen recorder
Laser blood flow monitor MBF2

monitored by a rectal thermometer.

2. Surgical procedures for stimulating and recording

(i): Saphenous nerve dissection

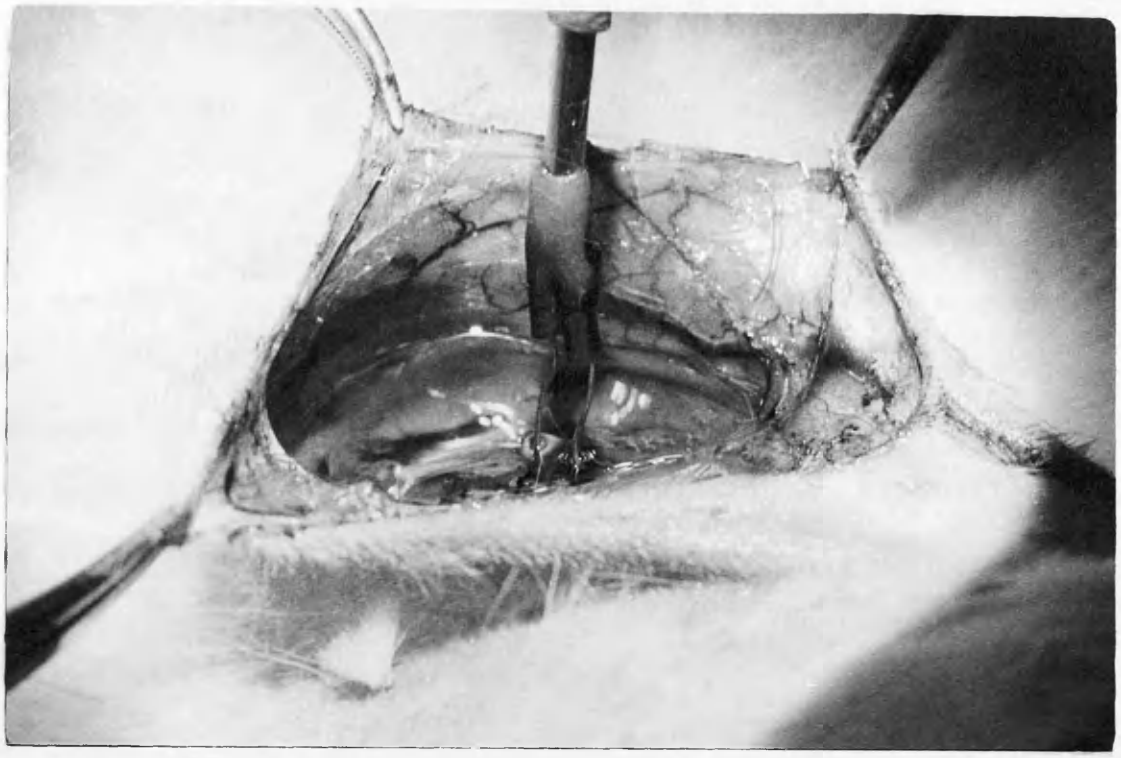
After the removal of fur from the antero-medial aspect of the thigh, an incision was made near the groin down to few centimeters close to upper part of the knee (Figure 2.11).The skin flaps were freed by blunt dissection and retracted, exposing the gracilis, sartorius,and rectus femoris muscles. By separating the gracilis and sartorius muscles, the saphenous nerve and some of its branches which are accompanied by the femoral artery and vein could now be seen. The saphenous nerve was then dissected free from the accompanying blood vessels, and the proximal end was cut and all branches except those to the knee were sectioned.

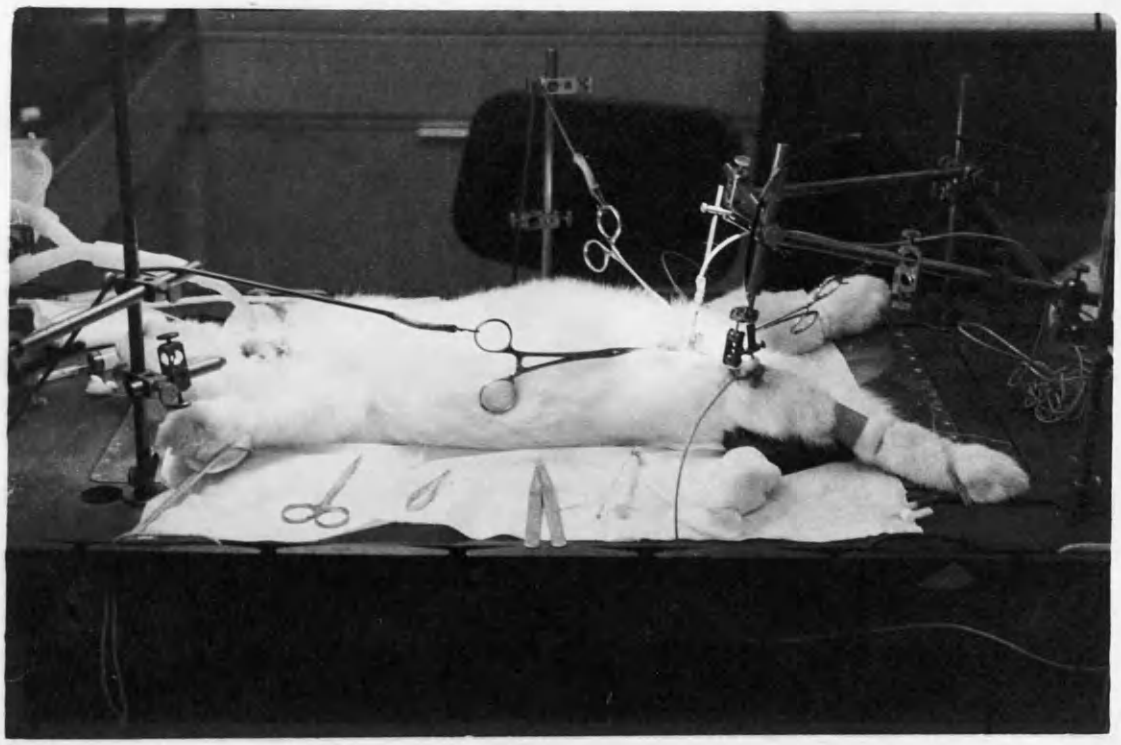
(ii): Thigh paraffin pool

After the dissection of the saphenous nerve, the thigh was held at a fixed position. Threads were then passed through the skin flaps and were retracted on either side. They were then tied to the frame enabling warm paraffin oil to be poured into the cavity thus formed (Figure 2.11). The temperature of this pool was maintained at 37°C by heating lamp if necessary.

Fig. 2.11. Photograph shows the antro-medial aspect of rabbit thigh with a thigh paraffin pool.

Fig. 2.12. Photograph shows the location of infra-red laser probe on the surface of anterior capsule of experimental limb.





(iii): Locating stimulating electrodes

When the saphenous nerve was dissected free close to the groin, bipolar silver electrodes were then placed on the distal end of the nerve and the stimulator (Harvard advanced stimulator) was set to deliver trains of rectangular pulses with different durations, intensities and frequencies.

(iv): Locating recording probe

To monitor relative changes in blood flow either a near infra-red (780nm) laser Doppler flowmeter MBF2 (Moor Instruments) or laser Doppler flowmeter MBF3 (Moor Instrument) with the capability of having three different wavelenghts (green, red, and infra-red) to penetrate different layers of tissue to monitor blood flow, were used. The former laser probe was placed on the surface of the capsule* anteriorly and the surrounding tissue covered with cling film (Figure 2.12), and the latter laser probe was inserted from antro-lateral side of knee joint below patella into the joint cavity facing toward inside to detect changes in blood flow in the antero-medial capsule** of knee joint. Changing the

*: Synovium and infra-patellar adipose pad (Knight & Levick 1983)

**: Synovium and areolar fibrous tissue (Knight & Levick 1983)

wavelenghts from red to infra-red permits monitoring greater depth of tissue blood flow respectively.

To check whether intra-articular laser probe is also monitoring the blood flow in skin covering the joint capsule, an incision was made on skin covered medial part of the knee joint and a piece of black polythene film was located under it. Blood flow was then recorded before and after removal polythene film, no significant changes of flow was seen (Figure 2.13).

The signals from the flowmeters were recorded on one channel of a pen recorder. At the same time the arterial blood pressure and heart rate were recorded on the other channels. However the MBF3 device was also able to record flux changes on an inbuild chart recorder.

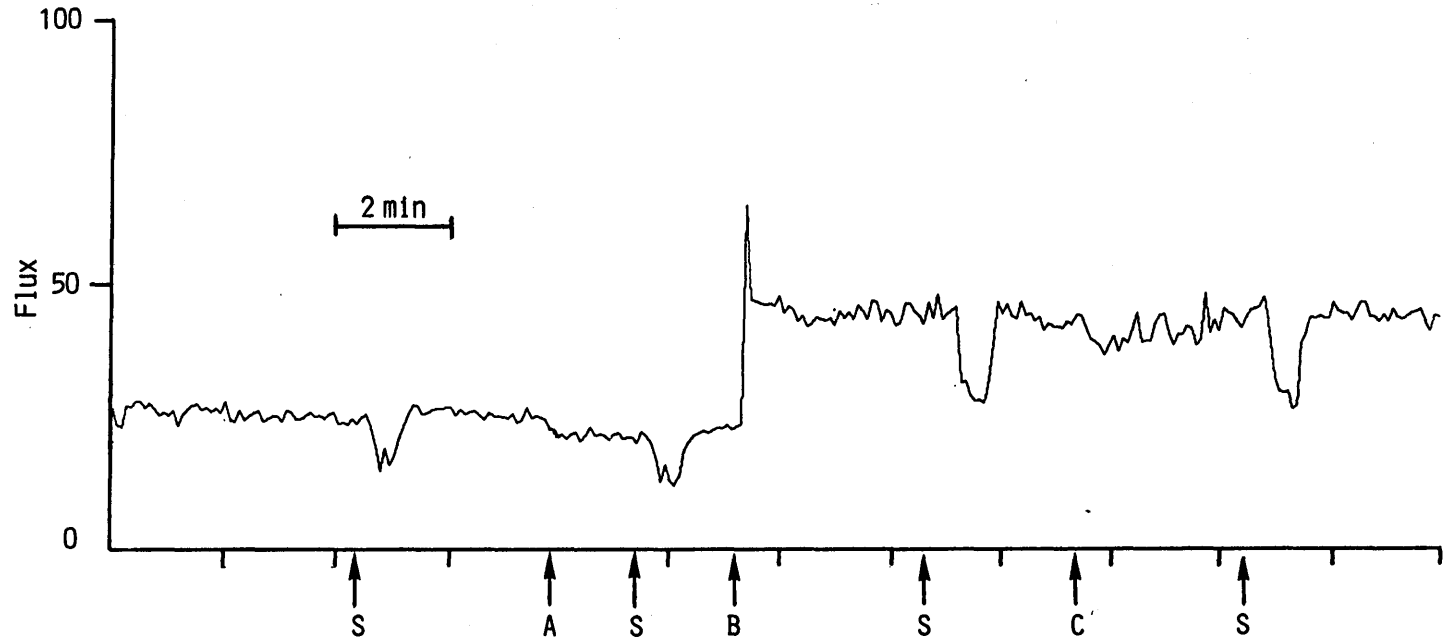
(v): Knee joint temperature monitoring

In some animals a 21G hypodermic thermocouple was inserted into the joint cavity and connected to an amplifier (thermalert TH-6D) to provide a measure of intra-articular temperature.

(vi): Termination of the experiment

At the end of the experiment rabbit was painlessly killed by an intra-arterial overdose injection of sodium pentobarbitone (Euthatal, May & Baker Ltd.).

Fig. 2.13. Trace showing the blood flow signal from a laser Doppler flowmeter monitoring blood flow by red laser light, (633nm) ,and at B using an infra-red (780-790nm) source. Arrow S represents onset of stimulation of the saphenous nerve for 30sec. At A, a black polythene film was interposed between the skin and knee joint, and at C, it was removed. Neither the flux signal (at either wavelengths) nor the magnitude of the constriction response were significant affected. This trace is the out put of MBF3 plooter and represents the running avaraged at 10 seç intervals.


100
50
0
Flux
2 min
S
A
S
B
S
C
S

III. Perfusion technique in the rabbit

After anaesthetizing the rabbit and all the surgical procedures which were carried out, to isolate the blood flow to one of the knee joint from the effect of systemic blood pressure changes, a perfusion technique was used.

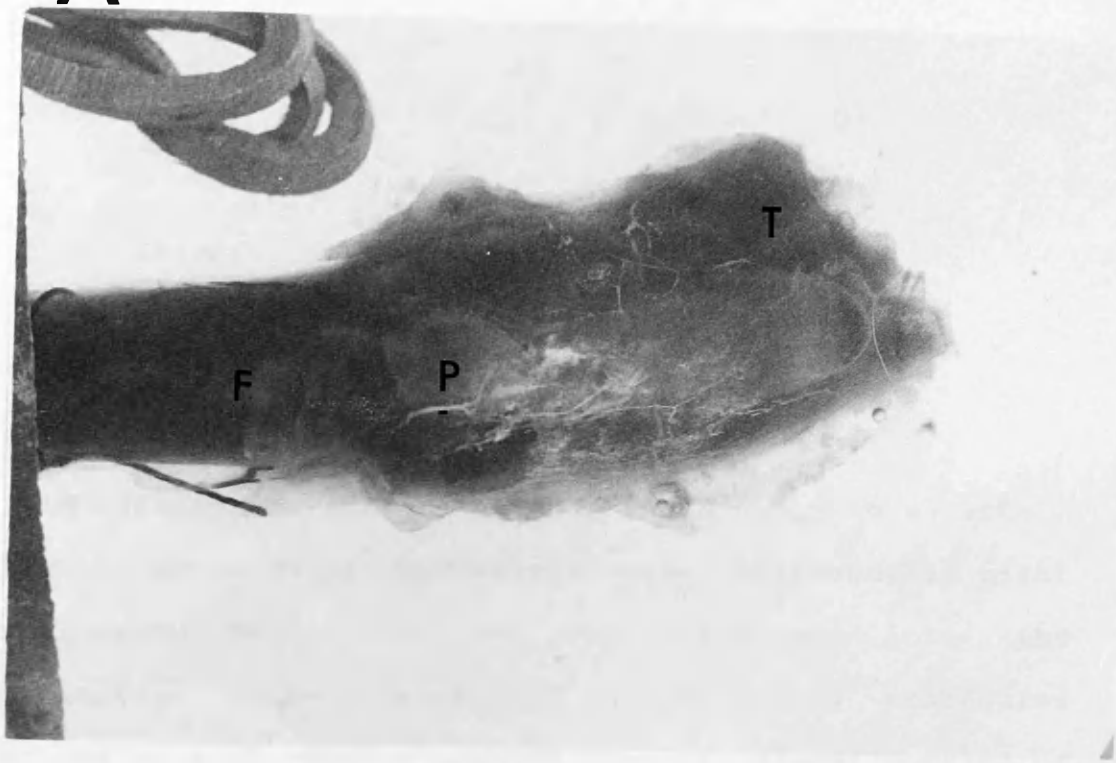
For this purpose, the femoral artery of the appropriate knee was cannulated and connected to the carotid cannula, and a Watson-Marlow pump was interposed to pump the blood from the carotid to the femoral at a constant rate and at the same time the perfusion pressure was measured by a pressure transducer connected "down stream" from the pump. To prevent further clotting of blood, the animal was heparinized.

IV. Injection of potassium iodide barium sulphate in-vitro.

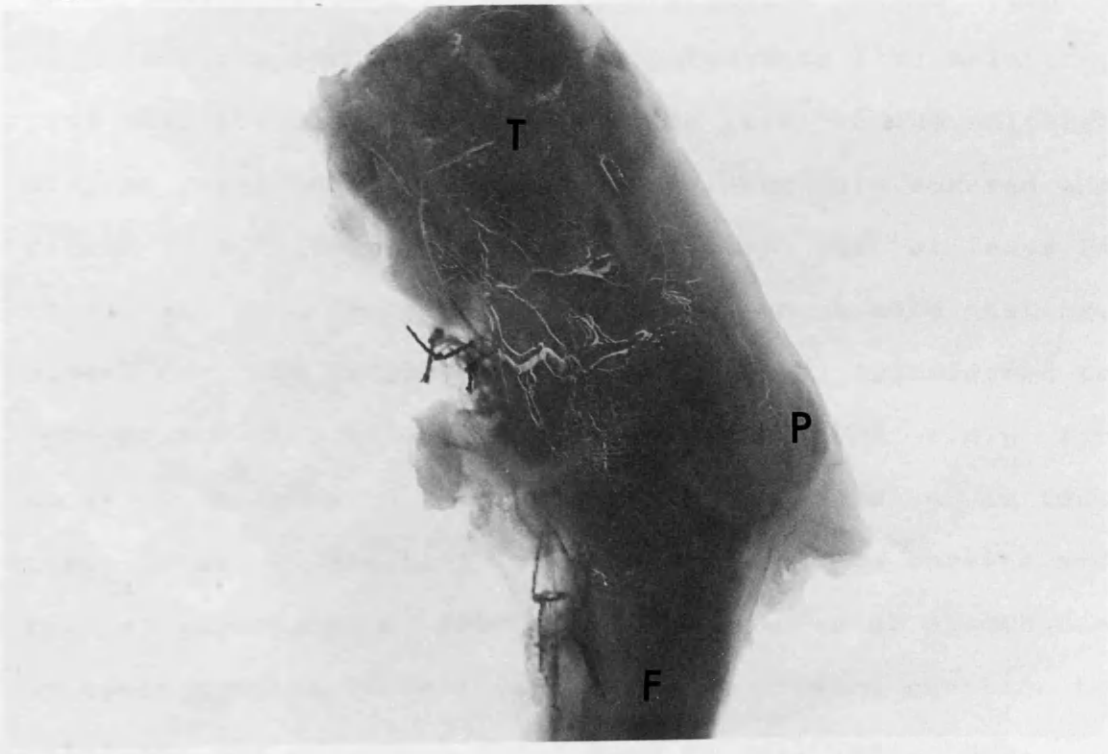
In order to check that just the joint blood vessels were perfused and not other surrounding tissues, in a few preparation at the end of the experiment injection of a radiopaque solution was used for radiographic visualization of articular blood vessels (Appendix B). As illustrated in figure 2.14, there was little radiopague in surrounding muscles but it is hard to judge about the bones (Femur and Tibia). Thus, another technique was used to find out whether blood vessels other than knee joint blood vessels were perfused during in-vitro experiments.

Fig. 2.14. Photograph of blood vessels containing the radio-paque (potasium iodide barium sulfate). A: Anterior aspect. B: Lateral aspect, showing that radiopaque is present in joint blood vessels. F: Femur, T: Tibia, P: Patella.

A



B



V. Injection of Evans blue in the rabbit

a. In-vitro

In addition to the radiopaque technique, to ascertain whether surrounding structures were perfused, in eight experiments Evans blue dye was injected into the perfusate. After five minutes the following procedures carried out; 1. Tissue samples from the distal portion of femur, proximal portion of tibia, surrounding muscles, anterior and posterior capsules from both legs were removed and weighed separately. 2. Tissue samples were cut into small pieces. 3. Each tissue sample mixed with a solution, composed of 6ml sodium sulfate (1%) solution, plus 14ml acetone in a 30ml bottle (i.e. sodium sulfate: acetone ratio was 3:7). 4. All containers were covered and placed in a Heidolph electrical agitator for at least 24 hours at room temperature with continuous mild shaking. 5.Next day, the extracted solutions were transferred to appropriate test tubes and centrifuged at 2000 r.m.p for about 10 minutes. 6. From the top of the solution in test tube, 3.6ml supernatant was transferred to cuvette and read at absorbance of 620 nm. 7. The reading of absorbance by spectrophotometer was put in the following equation to calculate the amount of Evans Blue in ug/100mg tissue.

$$\text{amount of Evans (ug/100mg tissue)} = \frac{\text{Absorbance X Gradient* X Volume of Solvent}}{\text{Weight of tissue X 10}}$$

*: Gradient was constant and was equal to 13.24, this number was achieved by converting the slope line on figure 2.15.

As it has been shown in figure 2.16, the dye (mean ± SEM microgram Evans blue/100mg tissue; n=8) was present in large quantities in the posterior capsule (48.96 ± 2.18) and to much smaller extend in anterior capsule (3.12 ± 0.27) but little was present in other areas (Muscle, 0.35 ± 0.1;Femur, 0.31 ± 0.11; Tibia, 0.23± 0.05). The values for the anterior capsule differed significantly from these other areas ($P<0.001$) and from the posterior capsule ($P<0.001$). These results indicate that the ligatures has successfully isolated the articular circulation.

b. In-vivo

As Evans blue binds to plasma proteins normally restricted to the vascular compartment, its presence in joint tissue provides an indirect means of assessing capillary permeability. To assess whether the permeability of articular blood vessels have been increased or not, Evans blue was injected intravenously into the ear vein in a dose of 75mg/kg. The technique

Fig. 2.15. The slope line for light absorbance (620 nm) of different concentrations of Evans blue (ug/ml) is shown. slope= 0.075, 1/slope = 13.24 (gradient).

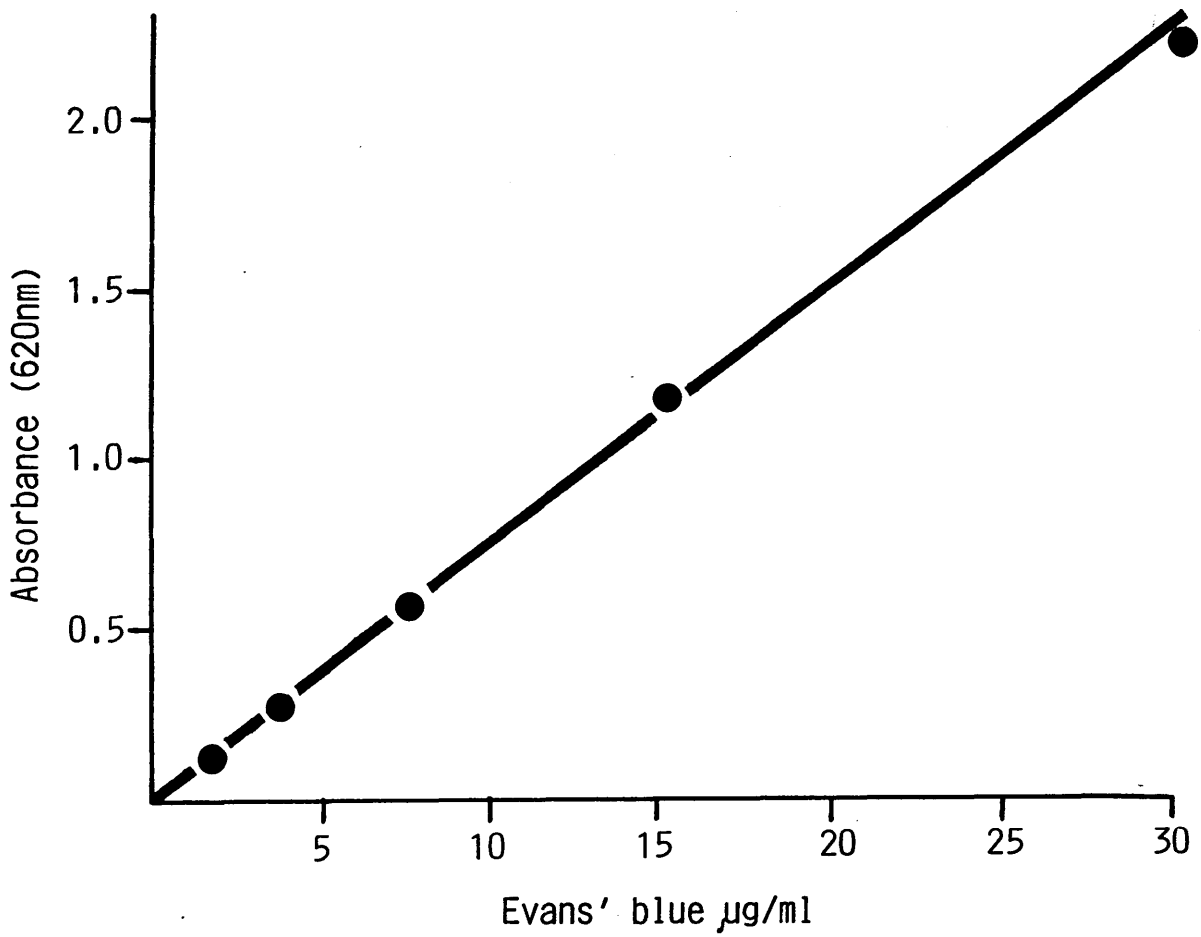
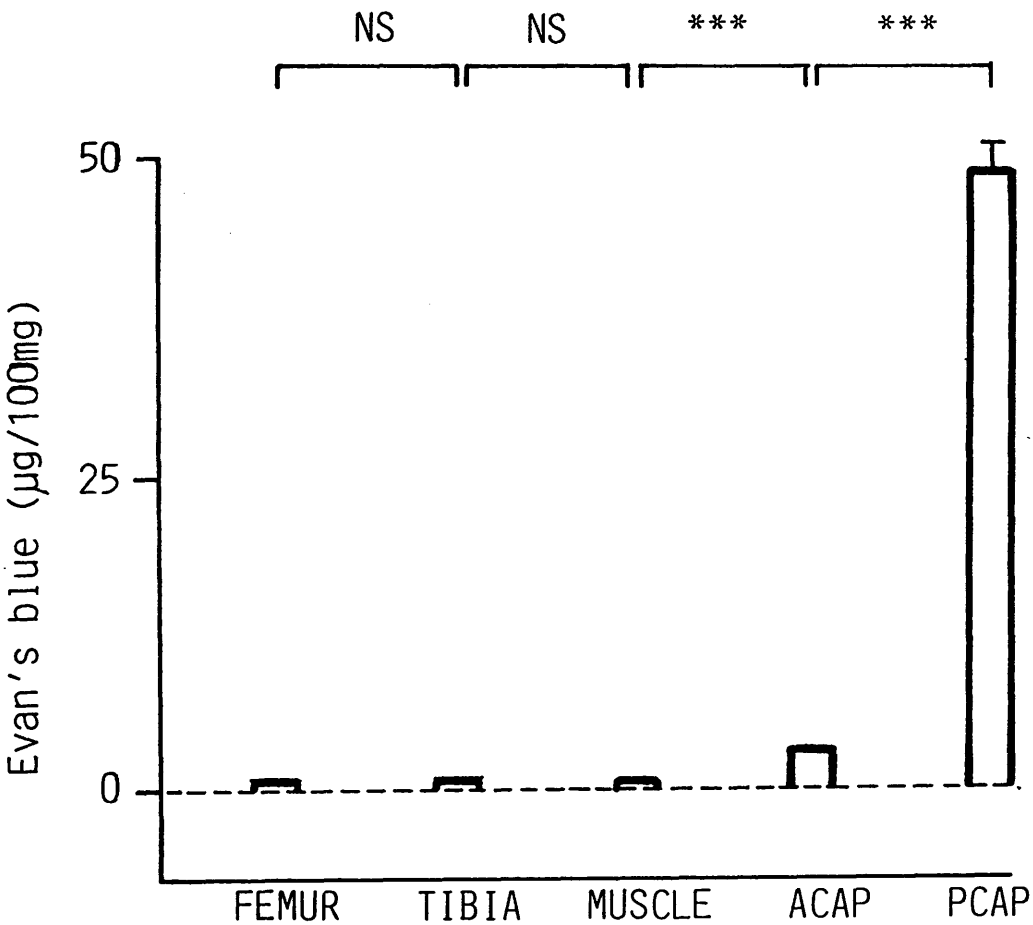

Absorbance (620nm)
2.0
1.5
1.0
0.5
5
10
15
20
25
30
Evans' blue µg/ml

Fig. 2.16. Evans blue content (mean ± SEM) of various tissues five minutes after perfusion. The highest concentration of dye was in the posterior portion of the capsule (PCAP), with less occurring in the anterior capsule (ACAP). Negligible quantities were found in other areas. *** $p<0.001$: $n = 8$.


NS
NS
***
***
50
25
0
Evan's blue (µg/100mg)
FEMUR
TIBIA
MUSCLE
ACAP
PCAP

used to extract Evans blue and its subsequent spectrophotometric measurements has been described above.

VII. Induction of acute joint inflammation

In some experiments, acute inflammation was induced in the rabbit knee by intra-articular injection of 2ml kaolin (4%) suspension (Sigma) into one knee joint while the other knee joint was injected with 2ml sterile physiological saline to act as an internal control.

The induction of acute joint inflammation was checked in the experimental knee by either monitoring intra-articular temperature compared to the control knee, or by measurement and comparison of Evans blue extravasation into the joint tissue of two knees.

C: Statistical analysis

Two types of Statistical data analysis were carried out, 1. Two way analysis of variance (ANOVA), and 2. Paired or unpaired t test. An F test was used to test the assumption of homogeneity of variances. Where this exceed tabled F values, modified t values were generated using the formula described by Phillips (1978). All data expressed on graphs are means $\pm$ standard error of mean (S.E.M.) . Differences between means were considered significant if the P values were 5% or less. *: $0.01 < P$

<0.05; **:0.001< P <0.01; ***: P<0.001.

****

****

****

****

*****

***

*

CHAPTER THREE

EVALUATION OF LASER DOPPLER FLOWMETRY IN THE ASSESSMENT OF ARTICULAR BLOOD FLOW IN CATS AND IN RABBITS

Summary

1. Experiments were performed in adult cats anaesthetised with pentobarbitone and in adult New Zealand rabbits anaesthetised with a mixture of hypnorm and diazepam plus halothane/N_2O/O_2.

2. A new method for the non-invasive and continuous measurement of articular blood flow is evaluated and introduced.

3. This technique is based on the fact that laser light is back scattered from the moving red blood cells, with Doppler shifted frequencies. This signal goes to photodetectors and is converted to a flow signal.

4. The laser Doppler flowmeter (Moor Instruments MBF2, MBF3) provided a measure of relative changes in blood flow.

5. To evaluate this technique, comparison was made between this and the radiolabelled microsphere technique.

6. Intra-ventricular injection of three types of radio-labelled microsphere with timed withdrawal of an arterial blood sample allowed measurement of blood flow after denervation and prior to insertion of probe into the joint and before and during nerve stimulation of the cat

PAN and the rabbit saphenous nerve.

8. These results indicate that; a. There is a high correlation between changes in arterial blood pressure and changes in flow meter signal ($r=0.992$, $P<0.001$, $n=8$). b. The percentage change in the laser flowmeter signal is significantly correlated with the percentage change in blood flow as measured with microspheres ($r=0.908$, $P<0.001$, $n=17$), therefore the laser Doppler technique provides a suitable means of assessing relative changes in joint capsular blood flow. c. Bones around the joint are also innervated by presumed sympathetic vasoconstrictor fibres travelling in nerves supplying the joint.

Introduction

The need to measure local blood flow in small volumes of tissue arises in many contexts in physiology pharmacology, and clinical medicine. Therefore, different techniques for measurement and estimation of blood flow through different organs and tissues have been tried. These techniques include, 1). Application of the plethysmograph to knee segment in human (Bonney et al. 1952), 2). An indirect measurement of temperature which represents change in blood flow to appropriate organs or tissue. e.g. monitoring intra-articular temperature in man (Horvath and Hollander 1949), in a animal such as cat the (Ferrell and Cant 1987), 3). Using magnetic or piezoelectric flow probe around the arteries (Busija et al 1981), 4). Clearance method drived from that of Kety (1949), 5). Measurement of arterial diameter (Haberl et al 1989b), 6). The radiolabelled microspheres technique (Christensen et al 1982,Bunger et al 1983, 1984), and 7). The laser Doppler flowmetry technique which has been used as reliable and accurate technique to measure blood flow of highly localized microcirculation of different tissue such as intestinal blood flow (Ahn et al 1985), cerebral blood flow (Busija et al 1981, Haberl 1989a,b), cutaneous blood flow (Holloway et al 1977), renal cortical and medullary blood flow (Bowman and Stein 1979),bone blood flow (Hellem et al 1983, Swiontkowski et al 1986, Notzli

et al 1989), retinal blood flow (Riva et al 1972) and articular blood flow (Khoshbaten and Ferrell 1989).

The clearance technique is based on the principle that the rate of disappearance of a known substance e.g. H_2 (a highly diffusible, biologically inert gas) from perfused tissue is determined by the blood flow (Aukland et al 1964). This technique offers the advantage that it provides reasonable quantitative estimates of blood flow but the disadvantage of this method is that, it can do so only on an intermittent basis.

Techniques such as plethysmography and temperature monitoring, although capable of continuous recording of changes in blood flow, may not precisely reflect the events in small and limited vascular beds.

Diameter measurements can provide a extremely localized estimation of changes in blood flow. But this technique is not able to show exactly changes in other parts of the arterial tree. By this method, measurement can be made in a few seconds, so that both transient and steady-state responses can be examined, but this is still cumbersome to perform on a continuous basis.

Flow measurement by magnetic or piezoelectric flow probe, can both provide a continuous measurement of blood flow but this is difficult to perform and is sometimes associated with arterial spasm at the probe location (Busija et al 1981).

The radiolabelled microspheres technique was

introduced by Rudolph and Heymann (1967), to measure regional blood flow. These were injected into the circulation and travel to the small peripheral vessels where they were trapped. The organs were then removed and their radioactivity measured. Repeated measurements could be made by using different nuclides and separating them by gamma spectrometry. This technique which is widely used by many investigators to evaluate other flow measurement techniques is capable of blood flow measurement in a quantitative manner. However, this technique is also limited to a discontinuous measurement and can not accurately measure flow changes in a highly localized microcirculation which consists of only a few cubic millimeters.

Consequently, by reviewing different techniques as mentioned above, it seems that there is still a need for a technique that is easy to handle, permits instantaneous and continuous monitoring of the blood flow with least damage to the tissue under study beyond the requirement of exposure of the tissue, good localization, and also allows measurement of relative changes in regional blood flow. The technique which is most suitable to show a potential capacity to fulfill these criteria, could be the Laser Doppler flowmetry method. Laser Doppler flowmetry provides easy to use, non-invasive, real time measurements of local tissue blood flow. Laser Doppler flowmetry (LDF), was first used for blood measurement in single vessels (Riva et al 1972) and later used by Stern (1975) to measure

skin blood flow in humans. It is also reported to have been used to assess blood flow measurement in different tissue such as renal cortex and medulla, brain, and intestinal mucosa (Holloway and Watkins 1977, Stern et al 1979, Busija et al 1981, Ahn et al 1985).

LDF is based on the principle that coherent light scattered by moving red blood cells experiences a frequency shift that is proportional to the number and velocity of red blood cells flowing through the microcirculation (Nilsson et al 1980, Bonner et al 1981, Haberl et al 1989a). The fibre optic guides, which conduct the laser light from the laser source to the tissue and carry back scattered light to a photodetector, are placed on the appropriate tissue surface for continuous measurements of regional blood flow. To find out a closer relationship between the microcirculation in-vivo and the laser Doppler signal, it is necessary to examine a comparison between this method with other techniques measuring blood flow. Blood flow measurement by LDF has been found to correlate linearly with flow measured by other techniques, including hydrogen (H_2) clearance (Haberl et al 1989b), ^{133}Xe washout and electromagnetic flowmeters (Engelhart and kristensen 1983, Kvietys et al 1985), arteriolar diameter change (Haberl et al 1989a,b). Eyre and his colleagues have recently shown good correlation of LDF and microsphere measurement of cerebral blood flow (Eyre et al 1988). In a more recent study LDF was used with two other established techniques of H_2

clearance and observation of pial arteriolar diameter in brain of cat and rabbit to assess the LDF technique (Haberl et al 1989a,b).

At present there is relatively little quantitative data about knee joint blood flow, and those factors influencing the distribution of blood flow around joints. Different techniques to measure articular blood flow have been reviewed (Liew and Dick 1981, Levick 1984). As discussed quoted in a recent review paper by Levick (1987), the most popular technique which is applicable to man is the clearance method. With this technique, quantitative estimation of blood flow to the joint has been measured by the rate of disappearance of rapidly-diffusing radiolabelled solute (e.g. ^{132}Xe, ^{23}Na, ^{131}I) from the joint cavity (Harris and Millard 1956, Harris et al 1958, Dick et al 1970, Wallis et al. 1985). However the disadvantage of this method is that it is unable to provide continuous measurement of articular blood flow, and it can only do so on an intermittent basis. Another method which recently was used to measure blood flow of periarticular tissue (synovium and capsule) is the radiolabelled microsphere technique (Christensen et al 1982, Bunger et al 1983, 1984). With this technique the number of microsphere counts in capsular tissue provided an estimate of blood flow. Capsular blood flow calculated by this technique gave values of 1.1 ± 0.5,and 0.8 ± 0.5ml/min/100g of right and left joint capsules respectively in dogs (Bunger et al 1983), 9.46 ± 3.36

ml/min/100g of hip joint capsule in puppies (Lucht et al 1983), and in another study, blood flow in canine articular tissue was reported to be 2.6ml/min/100g (Simkin et al. 1986). Radiolabelled microspheres used in measurement of blood flow through articular blood vessels also provide a quantitative calculation of flow, but it only offers discontinuous measurement and it is difficult to measure blood flow changes in a highly localized microcirculation supplying only a few cubic millimeters. So, in comparison to the more recent LDF technique, The microsphere method, which is widely accepted by many investigators, measures flow in a tissue volume that is greater than that measured by LDF and therefore unlike LDF does not represent flow in the same highly localized microcirculation. Another contrast between these two techniques is the advantage of continuous flow measurement by LDF technique but not microsphere technique. According to the present evidence, although LDF technique was recently used in assessment of relative changes in articular blood flow (Geboreck et al 1989, Khoshbaten and Ferrell 1989), there is no thorough validation of LDF method for use in the knee joint in the existing literature. Therefore attempts were made to evaluate the use of LDF in articular blood flow measurement by studying the correlation between LDF and radiolabelled microspheres.

For physiological studies of knee joint blood circulation, however, each of these techniques has one or more of the following limitation: extensive surgery may be

required, discontinuous measurement, lack of quantitative calculation, less physiological conditions, and unable to monitor highly localized flow of joint capsule. So the main purposes of these experiments are: 1) To report upon studies with an instrument that demonstrates the feasibility of measuring articular blood flow by LDF. 2) To determine reliablity of the LDF method in assessing articular blood flow by comparison with an accepted method for blood flow measurement,i.e. the radiolabelled microsphere technique. 3) To compare changes in articular blood flow during electrical stimulation of the nerve supply to the knee with blood flow of adjacent tissues such as femur, tibia and popliteus muscle.

EXPERIMENTAL PROTOCOL

1. Method

Experiments were performed in 10 adult cats of either sex (2.4-4.5 kg), and 10 adult New Zealand rabbit of either sex (2.5-4.3 kg). Cats were anaesthetised with pentobarbital sodium (45mg/kg ip) and rabbits were first injected with hypnorm (0.1mg/kg im) and diazepam (0.5mg/kg ip). Anaesthesia was sustained in the cat by supplementary doses of pentobarbital sodium (intra-arterial) as required and in the rabbit, a gaseous anaesthetic was administered (2% halothane in O_2/N_2O). The animals were maintained deeply anaesthesia as judged by the absence of a flexor withdrawal response to noxious stimuli applied to the forelimb. The trachea was intubated to facilitate breathing and as a means of inspiration of halothane/O_2/N_2O mixture in rabbits. The surgical preparation used in these studies was as follows: In both animals, cannulation of the right carotid artery was performed to withdraw reference blood samples and monitor arterial blood pressure prior to and immediately after withdrawal of reference blood samples. In some experiments, arterial blood samples were taken in order to measure PO_2, PCO_2 and pH. Typical values were 110-118mmHg, 31-33mmHg and 7.398-7.410 respectively. Another cannula was inserted into the left carotid artery and advanced into the left ventricle for microspheres

in diameter (15μm) injection. The presence of the cannula tip in the ventricle was confirmed by recording the left ventricular pressure.

Since the use of LDF for measurement of blood flow is a novel technique, the principle of operation will be briefly reviewed. The MBF3 (Moor Instruments) laser Doppler flowmeter has the facility to emit either an infra-red (780-790nm), or red (633nm), or green (543.5nm) wavelength that is directed to the tissue through an optical fibre. Thus any of these could be used without dislocation of the laser probe was necessary. As light enters the capsular tissue, photons are scattered in a random fashion by moving blood cells and also stationary tissue cells. Because of random scattering events, directional blood flow is not a measurement parameter. Only blood concentration and blood velocity are determined. Blood flow is computed by determinig the product of blood concentration and blood velocity which appears as flux. Photons that interact with moving blood cells are Doppler (frequency) shifted and scattered. Photons that interact with that stationary cells are also scattered but not Doppler shifted. A portion of the scattered light which is reflected back into another fibre in the probe, and return this light to a photodetector. From the photodetector an electrical signal generates as flux and this signal from the photodetector contains frequency and power information, the former represents the blood cell velocity, whilst the latter is related to blood

concentration. So, as a result, the laser Doppler flowmeter is able to provide continuous measurement of relative changes in blood flow.

In each animal right hindlimb was left intact to provide a control knee joint whilst the other (experimental) limb was denervated proximally. Dissection and denervation of posterior articular nerve in cat and saphenous nerve in rabbit were carried on as described in other sections (chapter 2, and chapter 5). A 0.9mm diameter fibre-optic probe was inserted into the synovial cavity via a 18G hypodermic needle. In the cat, the probe was inserted through the antero-lateral aspect of the knee and advanced posteriorly until it made contact with the synovium of the postero-medial capsule. In the rabbit the probe was also inserted through the same region but advanced through the infra-patellar region until it made contact with the internal surface of the antero-medial capsule. After a clear and constant flow signal was achieved, the flow probe was kept firmly in position. An indicator of a clean flow signal was the pulsatile variation of flow when averaged with a 0.5-sec time constant. For the remainderof the experiments a 1 or 3-sec time constant was used to avoid this pulsatile fluctuation. The flux output as blood flow was monitored among with arterial blood pressure and heart rate on a pen recorder. The external recorder output of the LDF ranges from 0 to 5 volts full scale. Normal room illumination does not affect the LDF. However, in some experiments the

extremely intensive surgical dissection lamp could affect the base-line of LDF signal. Therefore, care was taken to turn off or redirect the dissecting microscope lamp before proceeding with flow measurement.

Radiolabelled microspheres ($1X10^6$-$2X10^6$ spheres) were injected in three different phases of experimental period, into the left ventricle over about 15-20 sec, and the injection was flushed with 3ml saline. Morris and Kelly (1980) reported that intra-ventricular injection of microspheres results in even distribution according to blood flow measurement of different tissues and no significant differences were observed in various arteries. The nuclides used were ^{57}Co, ^{113}Sn, ^{153}Gd,and ^{46}Sc.First injection was administered after cutting the nerve(PAN or saphenous nerve) and when arterial blood pressure was stabilized, The second injection was after inserting the probe into the joint cavity but before nerve stimulation, and the third injection was during electrical stimulation of nerve supply the knee joint when the maximum constrictor response was observed in the LDF signal. Microsphere with three different isotopic labels were injected into each animal in a random sequence. Withdrawal of reference blood sample was begun into a heparinized syringe just prior to injection of microspheres and continued for about 1 minute. The rate of withdrawal was 3ml/min in both species.

After about 10min of completion of last injection,

the animal was killed with an intra-ventricular injection of either sodium pentobarbitone (Euthatal) 200mg/kg or 3M KCl. Immediately after death, posterior, anterior knee joint capsules, 1cm distal part of femur, 1cm proximal part of tibia and popliteal muscle from both sides along with both kidneys were removed, weighed and placed in counting vials. In some experiments from both species of animals 1cm of distal part of humerus, 1cm of proximal part of ulna, cortical portion of femoral diaphysis, and femoral bone marrow were also removed, weighed and placed in counting vials. All tissue samples among with reference blood samples were then counted by a gamma counter. Nuclide separation was performed using differential spectroscopy by the method of Rudolph and Heymann (1967).

Tissue blood flow was calculated using the equation:

$$TBF = C_x \times 100 \times RBF / C_T$$

Where TBF is tissue blood flow in ml/min/100g, C_x is counts per gram of tissue, RBF is reference blood flow (rate of withdrawal of blood samples from reference arteries in ml/min), and C_T is total counts in the reference blood samples.

Parameters of electrical stimulation of nerve supply to the knee joint were; 20 volts amplitude, 1msec pulse width, frequency of 30 HZ, and a train of almost 1 min duration.

Data in graphs are presented as means ± SEM. Analysis has been performed using non-parametric statistics; Friedman two-way ANOVA with the Mann-Whitney U test for analysis of two independent groups (control knee vs experimental knee) and Wilcoxon matched-pairs signed rank test for repeated measures of flow in the experimental knee (before and during nerve stimulation).

2.Protocol

The following experiments were designed to compare changes in articular blood flow and surrounding tissues (e.g. femur, tibia, and popliteal muscle) with the new technique, laser Doppler flowmetry and with microspheres in both cat and rabbit.

Articular blood flow was determined under steady-state conditions during control period in both hind limbs, and whilst articular nerve of experimental limb was electrically stimulated.

Results

1. Blood flow estimation by microsphere technique

a. Knee joint capsular blood flow.

The calculated data for blood flow estimates through anterior capsules and posterior capsules in both species (cat, n=8; rabbit, n=9) are presented in table 5 and 6.

In the experimental limb of the cat, posterior capsular blood flow increased after sectioning of the nerve supply to the joint (Figure 3.1B). At least 15 minutes after the insertion of laser probe into the joint cavity, there was significant rise in the anterior capsule blood flow in both species of animals (Figure 3.1A, and 3.2A). These results suggest that rise in capsular blood flow in the cat after the sectioning of the nerve (first injection) is due to denervation of blood vessels in posterior capsule, while the blood flow in the anterior capsule remains constant (Figure 3.1), because blood vessels in anterior capsule are mainly innervated by lateral and medial articular nerves (Freeman and Wyke 1966). These results also suggest that the elevation in anterior capsule blood flow is due to injury hyperaemia. The comparison between capsular blood flow in control leg of these two species of animals show greater flow in the rabbit capsules than the cat. There are

Table 5

Sample	Mean ± SEM (ml/min/100g)					
	1		2		3	
	n=4		n=8		n=8	
LAC	0.53	0.29	4.28	1.08	2.74	0.88
RAC	0.53	0.30	0.55	0.18	0.63	0.25
LPC	10.58	4.25	9.97	3.44	1.26	0.43
RPC	1.03	0.74	0.79	0.24	0.66	0.24
LM	4.15	0.86	3.44	0.33	3.43	0.86
RM	3.93	0.60	3.73	0.53	3.03	0.44
LF	13.98	2.29	14.70	4.01	6.18	1.59
RF	13.15	3.66	13.25	4.74	14.74	3.07
LT	11.25	2.11	12.12	3.49	6.95	1.61
RT	11.95	3.53	11.76	4.39	13.65	2.89
	n=4		n=4		n=4	
LFBM	44.00	5.85	42.06	6.28	40.57	3.22
RFBM	35.80	10.03	38.13	5.83	45.70	7.03
LFCB	1.60	0.60	1.17	0.41	1.50	0.55
RFCB	1.50	0.53	1.17	0.22	2.10	0.57
LU	6.50	1.82	7.10	1.97	11.20	2.50
RU	6.00	2.46	6.80	3.51	11.07	1.55
LH	10.90	2.87	10.83	3.96	14.63	4.64
RH	6.93	1.95	9.23	0.87	13.93	1.73
	n=4		n=8		n=8	
LK	245.43	134.80	181.95	54.20	183.90	32.58
RK	228.83	122.99	165.55	46.79	166.76	28.63

Table 5: Blood flow through different tissues were measured by microsphere technique in the cat after first (1), second (2), and third (3) injections. LAC;Left anterior capsule,RAC; Right anterior capsule, LPC; Left posterior capsule, RPC; Right posterior capsule, LM; Left poplitus muscle, RM; Right poplitus muscle, LF; Left femur, RF; Right femur, LT; Left tibia, RT; Right tibia, LFBM; Left bone marrow of femur, RFBM; Right bone marrow of femur, LFCB; Left cortical bone of femur, RFCB; Right cortical bone of femur, LU; Left ulna, RU; Right ulna, LH; Left humerus, RH; Right humerus, LK; Left kidney, RK; Right kidney. Right limb was used as a control limb.

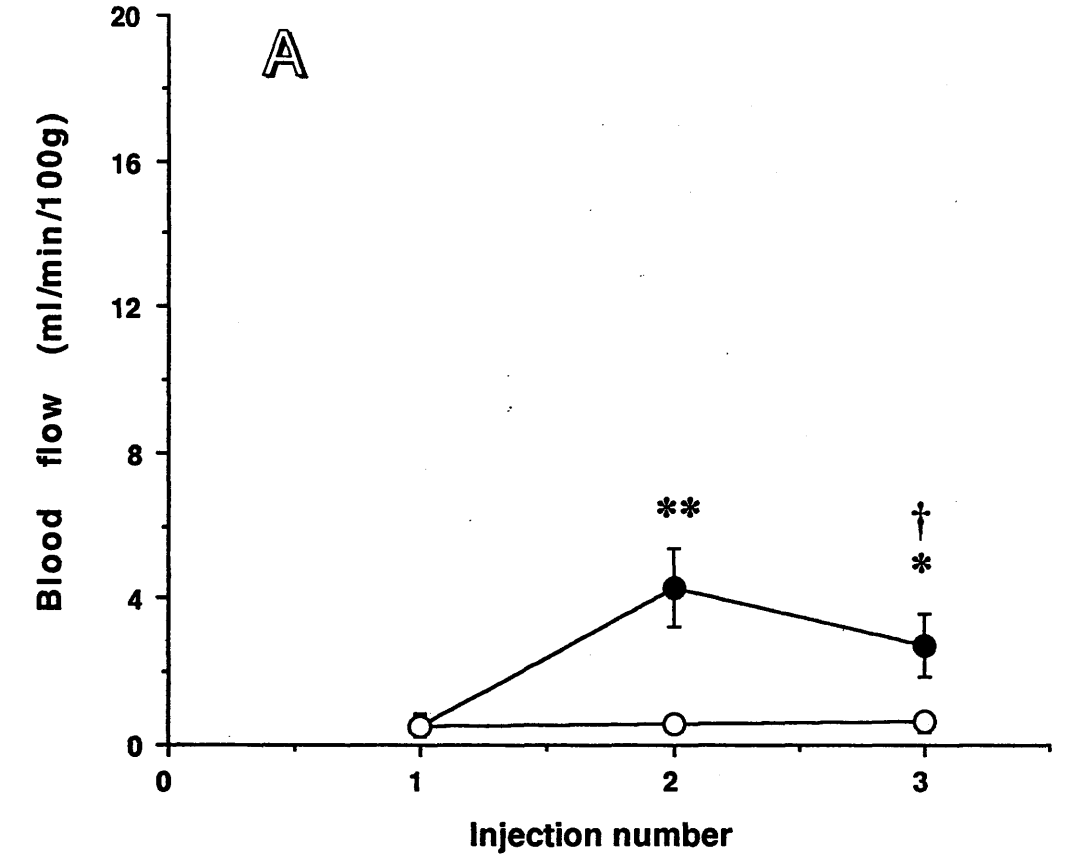
Table 6

Sample	Mean ± SEM (ml/min/100g)					
	1		2		3	
	n=5		n=9		n=9	
LAC	4.74	1.96	10.92	2.11	5.54	0.96
RAC	3.18	1.70	3.66	1.08	3.26	0.73
LPC	2.64	1.03	5.83	1.74	5.78	1.28
RPC	3.18	1.17	4.22	0.95	4.76	0.92
LM	5.32	0.98	5.83	0.49	6.04	1.06
RM	6.40	1.05	7.24	0.89	7.32	1.54
LF	12.88	3.49	13.23	2.74	9.90	2.31
RF	13.96	3.56	11.94	2.56	11.37	2.29
LT	14.32	3.99	13.33	2.71	10.24	2.11
RT	15.72	3.92	11.11	2.13	11.62	2.18
	n=5		n=5		n=5	
LFBM	34.28	6.42	31.54	7.33	38.92	6.51
RFBM	31.72	4.36	30.18	6.06	40.20	5.56
LFCB	5.52	1.23	4.94	1.19	4.28	1.18
RFCB	4.52	1.15	3.70	1.08	3.92	1.23
LU	13.33	3.03	10.83	2.80	14.75	5.13
RU	14.83	3.95	12.35	3.16	16.23	5.89
LH	13.35	1.60	10.48	4.48	10.00	2.08
RH	12.75	1.44	9.53	2.61	13.00	3.30
	n=5		n=9		n=9	
LK	187.14	18.84	223.67	39.68	229.73	35.65
RK	201.58	17.45	233.51	37.80	237.67	32.12

Table 6: Blood flow through different tissues were measured by microsphere technique in the rabbit after first (1), second (2) and third (3) injections. LAC;Left anterior capsule, RAC; Right anterior capsule, LPC; Left posterior capsule, RPC; Right posterior capsule, LM; Left poplitus muscle, RM; Right poplitus muscle, LF;Left femur, RF; Right femur, LT; Left tibia, RT; Right tibia, LFBM; Left bone marrow of femur, RFBM; Right bone marrow of femur, LFCB; Left cortical bone of femur, RFCB; Right cortical bone of femur,LU; Left ulna, RU; Right ulna, LH; Left humerus, RH; Right humerus, LK; Left kidney, RK; Right kidney.

Right limb was used as a control limb.

Fig. 3.1 The blood flow of anterior (A) and posterior (B) capsules measured in the cat. Numbers indicate the injection of different microspheres after sectioning of PAN (1), 15-20 minutes after insertion of laser probe into the joint cavity (2), and during the stimulation of PAN (3). Comparison were made between control (O) and experimental (●) legs. Values are means ± SEM. †,* $P<0.01$, **, †† $P<0.01$. *: comparison between experimental (●) and control (O) limbs, †: comparison between third and second injection.


A
Blood flow (ml/min/100g)
20
16
12
8
4
0
0
1
2
3
Injection number
**
†
*

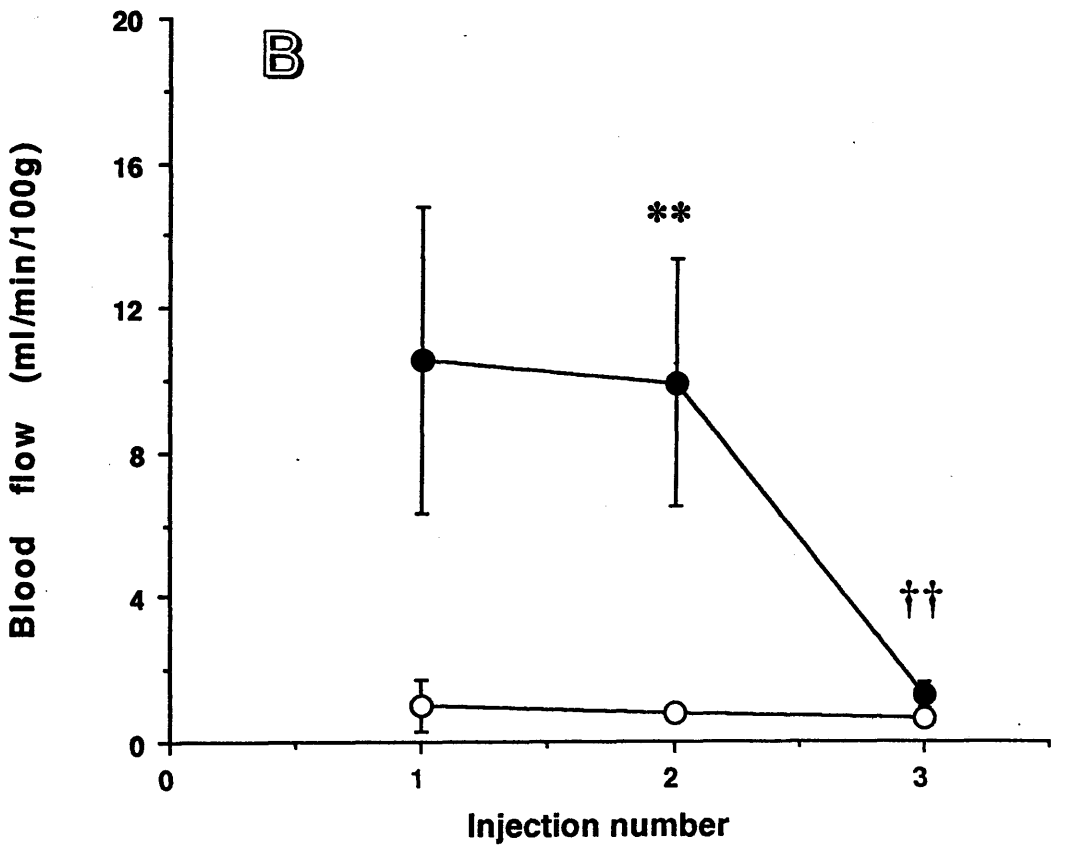
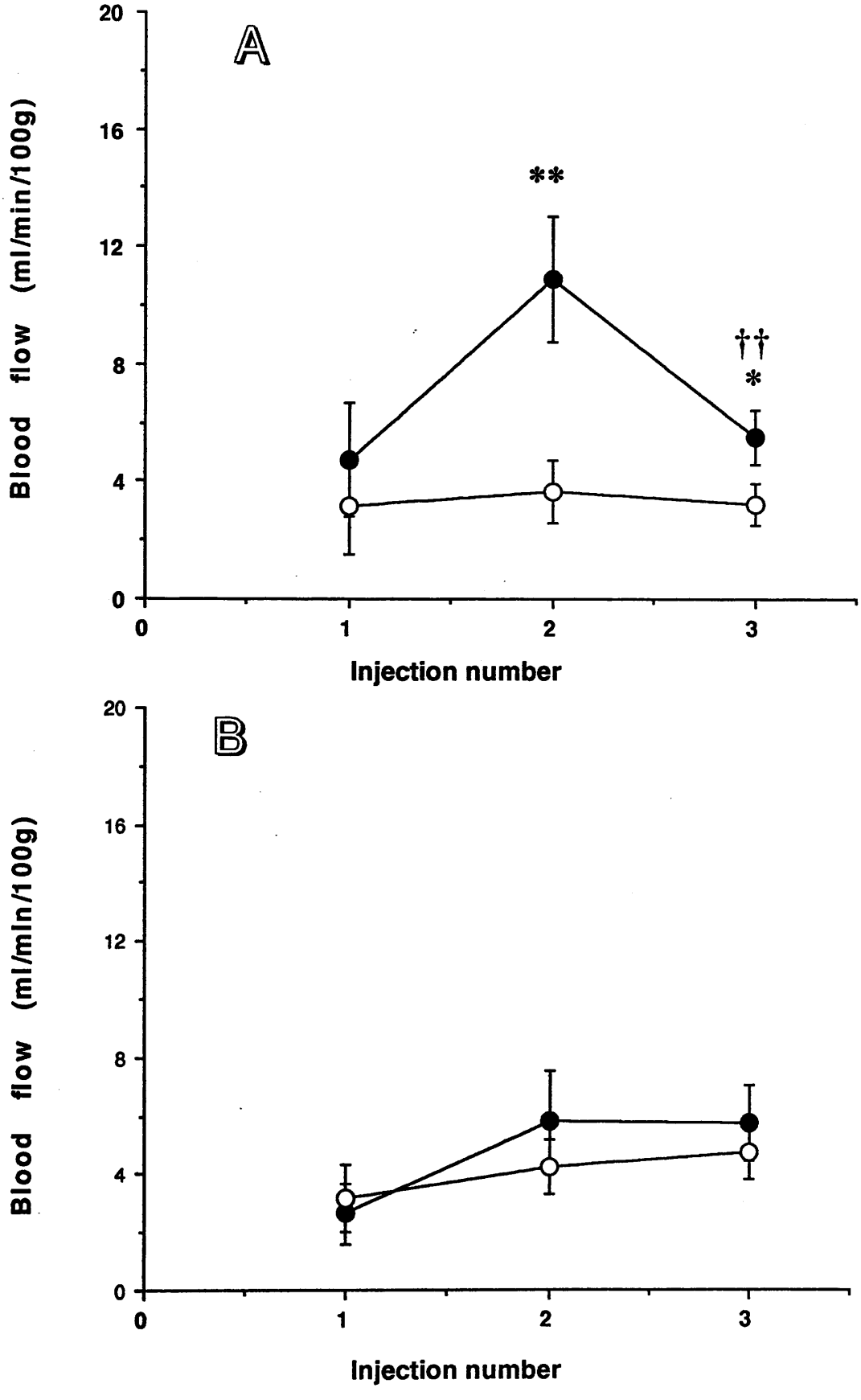

B
Blood flow (ml/min/100g)
20
16
12
8
4
0
0
1
2
3
Injection number
**
††

Fig. 3.2 The blood flow of anterior (A) and posterior (B) capsules measured in the rabbit. Numbers indicate the injection of different microspheres after sectioning of saphenous nerve (1), 15-20 minutes after insertion of laser probe into the joint cavity (2), and during the stimulation of saphenous nerve (3). Comparison were made between control (O) and experimental (●) legs. * $P<0.05$, **, †† $P<0.01$. *: comparison between exprimental (o) and control (O) limbs, †: comparison between third and second injection.


A
B
Blood flow (ml/min/100g)
Blood flow (ml/mln/100g)
Injection number
Injection number
**
††
*
0
4
8
12
16
20
1
2
3

possibilities that differences in capsular blood flow could be differences in species or different methods of anaesthesia, as halothane may have vasodilator effects on blood vessels.

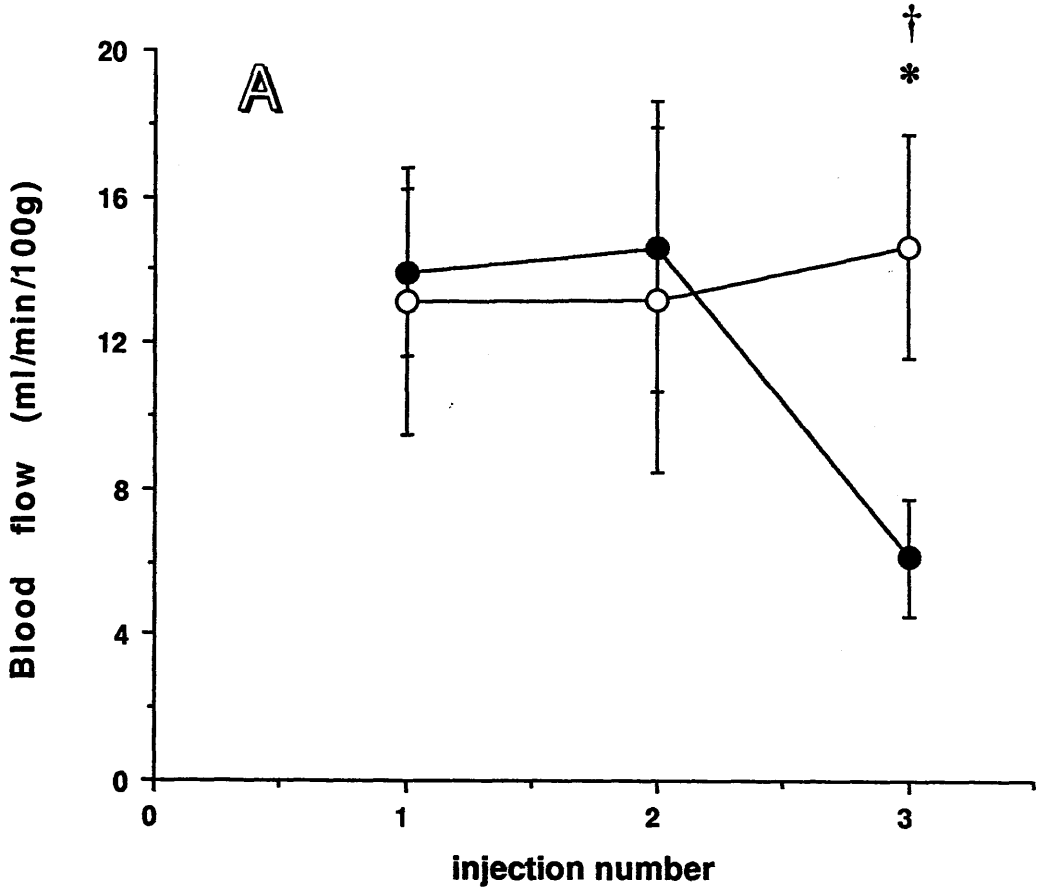
The stimulation of PAN induced significant reduction of posterior capsule blood flow in the cat (Figure 3.1B). This vasoconstriction is due to activating efferent sympathetic nerves as described in chapter 5. Also illustrated in figure 3.1A is that blood flow through the anterior capsule was reduced due to stimulation of PAN. This suggests that some of blood vessels in theanterior capsule are innervated by PAN.

b. Bone and popliteus muscle blood flow.

The data for blood flow estimates through bones and popliteus muscle close to the knee joint in both species of animals are presented in table 5 and 6.

As the results show, blood flow in cancellous part, bone marrow, and cortical part of femur, and cancellous part of tibia did not significantly differ in both legs neither in the rabbit nor in the cat after first and second injections. However in the cat there was a significant fall in blood flow of cancellous part of femur and tibia due to stimulation of PAN in the experimental limb but not in the control limb (Figure 3.3). This reduction in flow was less in the rabbit femur and tibia blood flow (Figure 3.4). As long as there is no significant differences in other tissues (Figure 3.5,

Fig. 3.3. The blood flow of the cancellous part of femur (A) and the cancellous part of tibia (B) measured in the cat. Numbers indicate the injection of different microspheres after transection of PAN (1), 15-20 minutes after insertion of laser probe into the joint cavity (2), and during the stimulation of PAN (3).*,† $P<0.05$. *: comparison between exprimental (●) and control (O) limbs, †: comparison between third and second injection.


A
†
*
20
16
12
8
4
0
Blood flow (ml/min/100g)
0
1
2
3
injection number

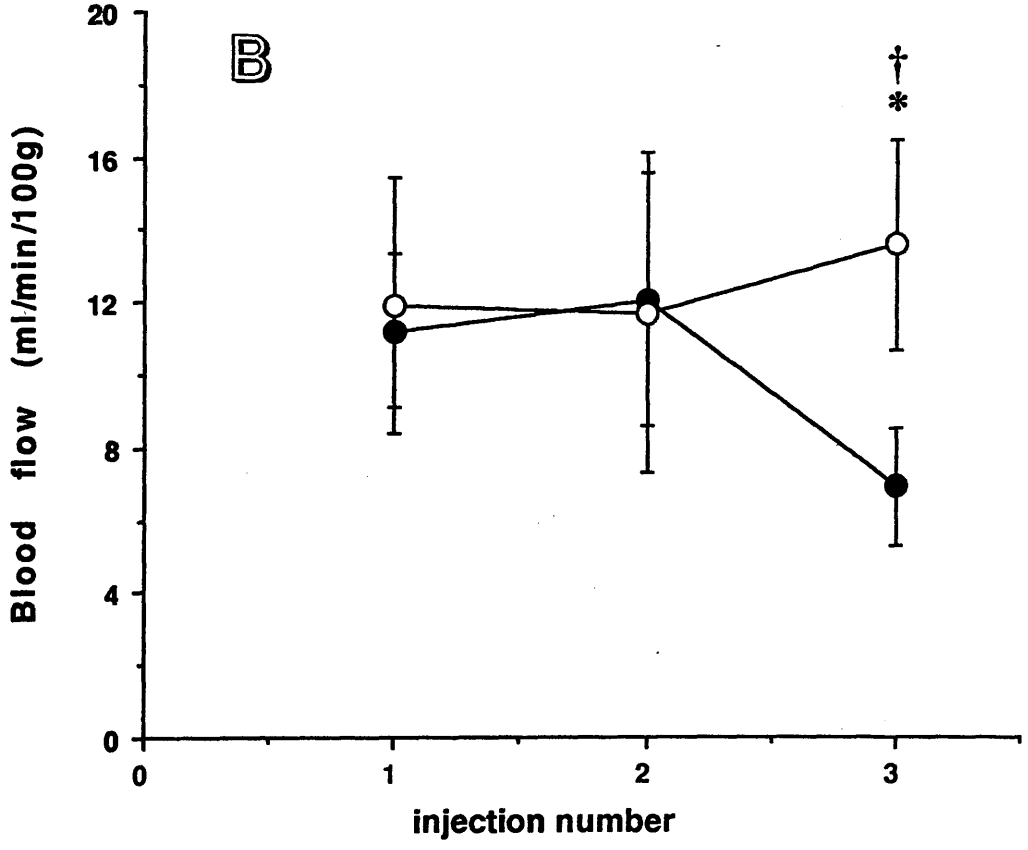

B
†
*
20
16
12
8
4
0
Blood flow (ml/min/100g)
0
1
2
3
injection number

Fig. 3.4. The blood flow of the cancellous part of femur (A) and the cancellous part of tibia (B) measured in the rabbit. Numbers indicate the injection of different microspheres after sectioning of saphenous nerve (1), 15-20 minutes after insertion of laser probe into the joint cavity (2), and during stimulation of saphenous nerve (3). **, †† $P<0.01$. *: comparison between exprimental (●) and control (O) limbs, †: comparison between third and second injection.

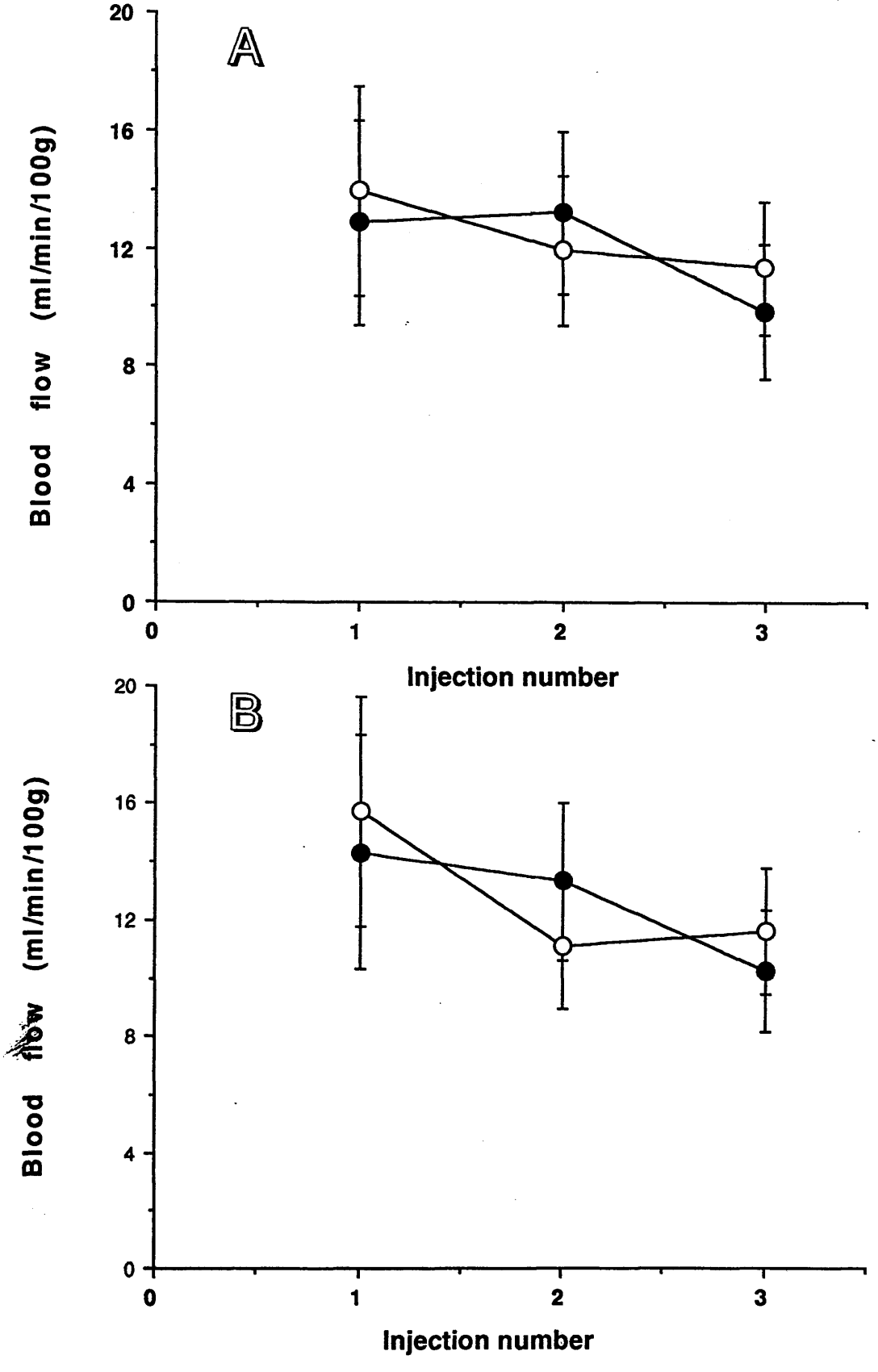
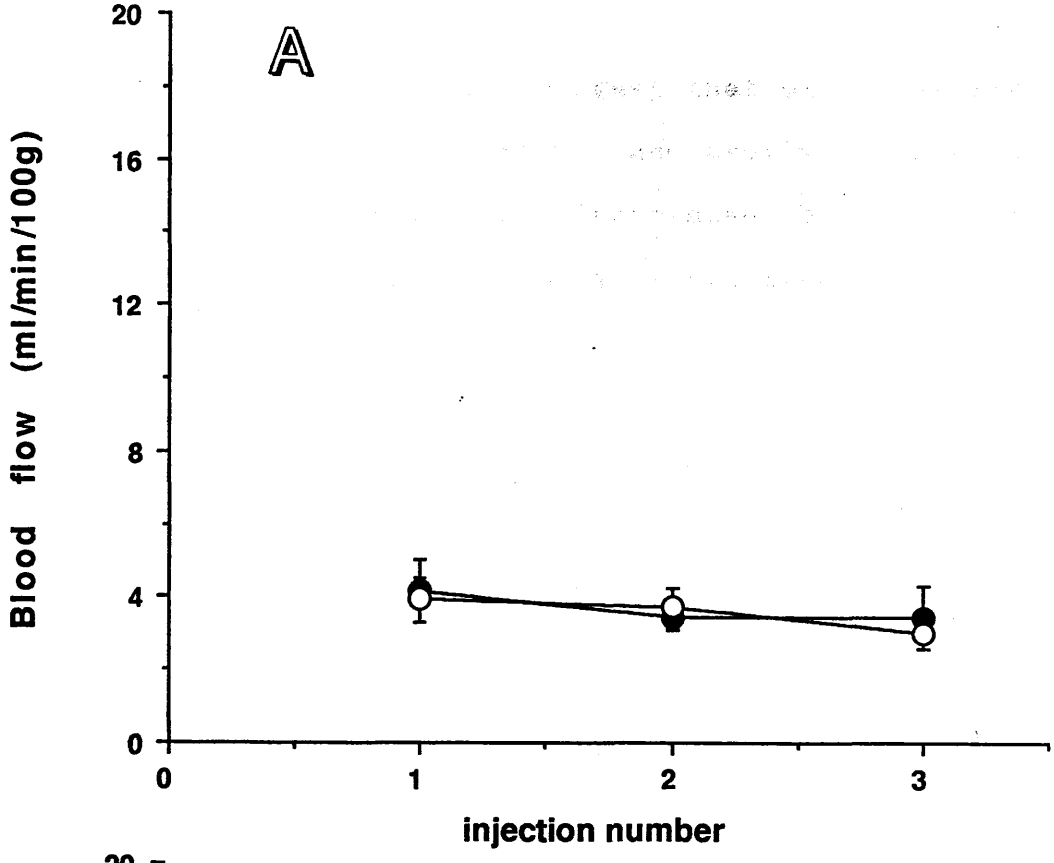

A
B
Blood flow (ml/min/100g)
Injection number
0
4
8
12
16
20
1
2
3

Fig. 3.5. The blood flow of the popliteus muscle measured in the cat (A) and in the rabbit (B). Numbers indicate the injection of different microspheres after sectioning of nerve supply th joint (1), 15-20 minutes after insertion of laser probe into the joint cavity (2), and during the stimulation of nerve supply the joint (3). Comparison between exprimental (●) and control (O) limbs.


A
Blood flow (ml/min/100g)
20
16
12
8
4
0
0
1
2
3
injection number

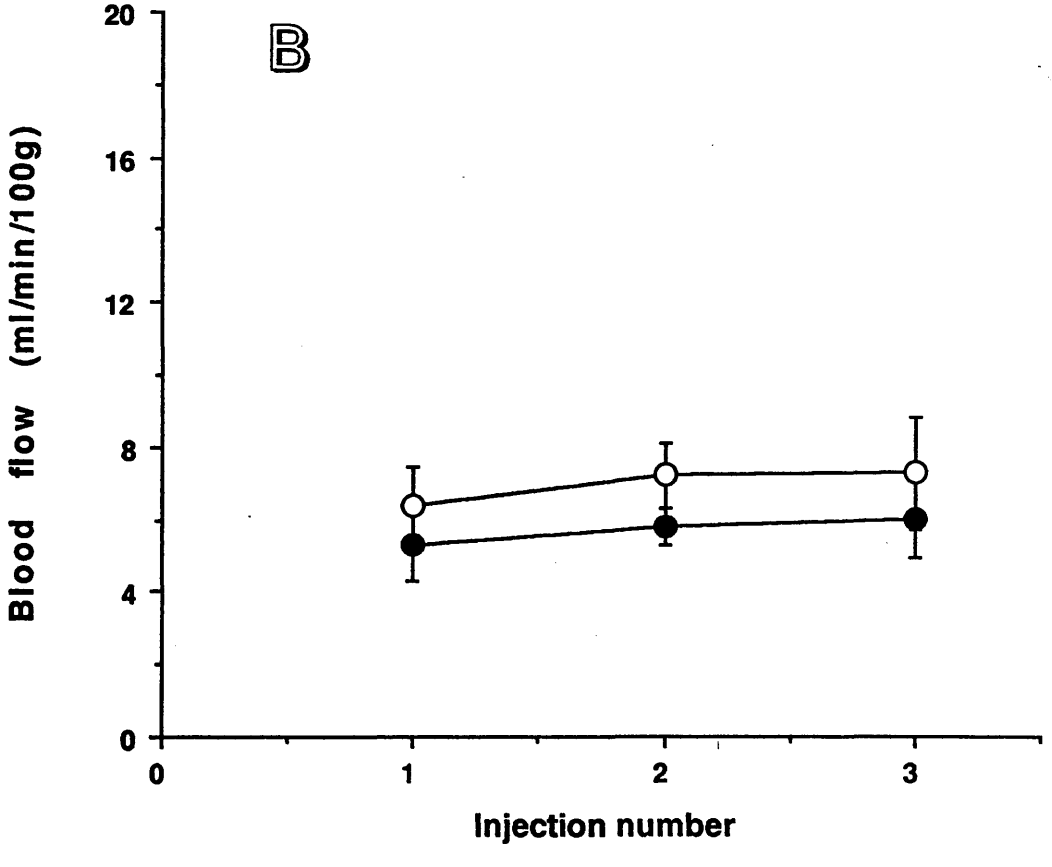
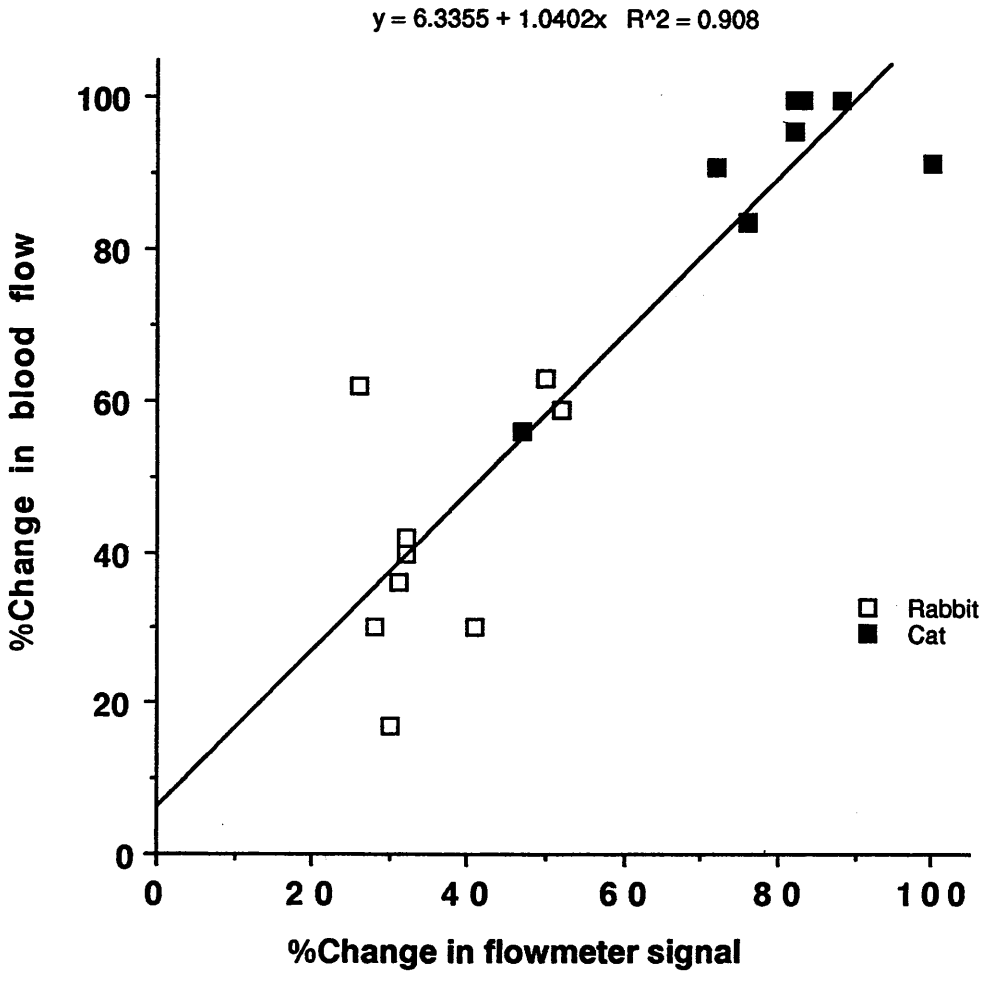

B
Blood flow (ml/min/100g)
20
16
12
8
4
0
0
1
2
3
Injection number

table 5 and 6), these results suggest that blood vessels in cancellous part of distal femur and proximal tibia in both species of animals are innervated by presumed sympathetic vasoconstrictor fibres travelling in nerves supplying the joint.

2. Correlation between the blood flow changes measured by LDF and microsphere techniques.

Electrical stimulation of the nerve supply to the knee joint (20V amplitude, 1msec pulse width, 30HZ train of about 1min duration) resulted in reduction of capsular blood flow in both species of animals. As illustrated in figure 3.6, the percentage changes in the laser signal were plotted against the percentage changes in blood flow as measured with the microspheres. The slope of this relationship is nearly one (1.0402). The slope and the correlation coeficient ($r= 0.908$, $P<0.001$, $n=17$) indicates that there is significant correlation between these two techniques for monitoring articular blood flow changes. Looking at the two groups, the change in the laser flow signal and blood flow was well correlated in the cat ($r=0.83$) but in the rabbit the correlation was poorer ($r= 0.378$). More significant correlation might be obtained by increasing the number of experiments. Overall, the laser Doppler technique appears to provide a suitable means of assessing relative changes in articular blood flow.

Fig. 3.6. The graph demonstrates the correlation between the percentage changes in blood flow measured with microsphere technique and percentage changes in flowmeter signal in both species of animals, cat (●)and rabbit (◘)

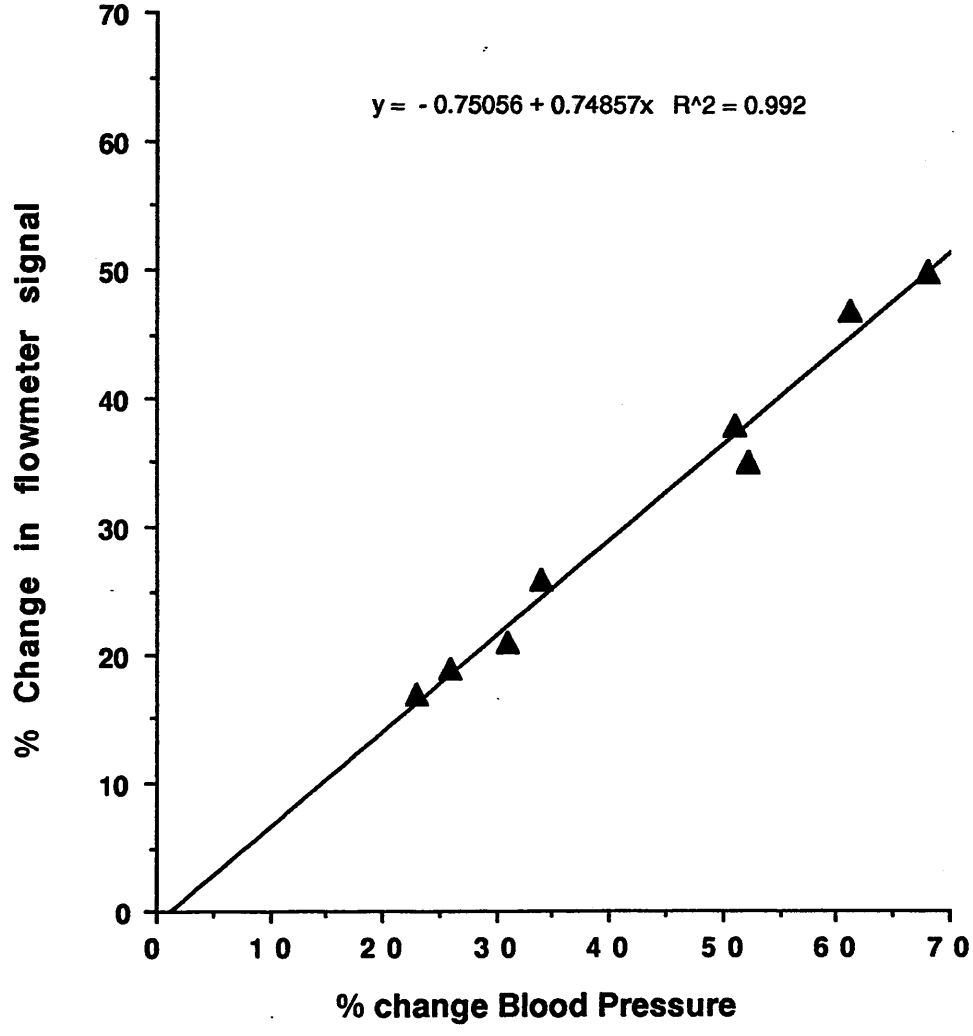

y = 6.3355 + 1.0402x R^2 = 0.908
%Change in blood flow
%Change in flowmeter signal
0
20
40
60
80
100
Rabbit
Cat

3. Correlation between changes in laser flow signal and blood pressure.

In order to check whether correlation exists between the flowmeter signal and arterial blood pressure*, in some experiments the percentage reduction in arterial blood pressure was calculated and plotted against the percentage changes in laser flow signal (Figure 3.7). The results show that, there is significant correlation between changes in the laser flow signal and blood pressure ($r=0.983$, $n=8$). This finding is comparable to results obtained by Shepherd and Riedel (1982). They showed a highly significant relation ($r=0.992$, $P<0.001$) between the percentage changes in perfusion pressure and percentage changes which occurred in the laser Doppler signal.

*: Reduction in arterial blood pressure happened randomly.

Fig. 3.7. Correlation between the percentage changes in arterial blood Pressure and the percentage changes in laser flowmeter signal monitoring articular blood flow. in either cat or rabbit n=8.


y = - 0.75056 + 0.74857x R^2 = 0.992
% Change in flowmeter signal
% change Blood Pressure
0
10
20
30
40
50
60
70

Discussion

The major contributions of this study are 1) use of a method that provides virtually continuous measurements of changes in articular blood flow, 2) actual blood flow in knee joint of cat and rabbit, 3) whether stimulation of nerve supply the knee joint blood vessels alter blood flow to other surrounding tissues such as bone and muscle. The discussion will focus on 1) consideration of methods, 2) Laser Doppler Flowmetry and correlation with measurement of articular blood flow with microspheres, 3) effect of articular nerve stimulation on blood flow of knee joint ,femur, tibia, and popliteal muscle. and 5) application of the LDF technique.

Method

Several techniques for measuring articular blood flow have been used. Amongst them, two techniques are most popular. One which is also applicable to man, is clearance technique(Harris and Millard 1956, Harris et al 1958, Dick et al 1970), and second one is the radio-labelled microsphere technique (Chrestensen et al 1982, Burger et al 1983,1984). In several recent studies and reviews the potential advantages and limitation of these techniques have been addressed(Buckberg et al 1971, Kvietys et al 1985, Levick 1987, Haberl et al 1989a,b). The clearance technique offers certain advantages permitting a

quantitative flow measurement which would otherwise be impossible in-vivo. Although the technique is simple, it requires accurate placement of the isotope and careful injection. The main disadvantage to this method is discontinuous blood flow monitoring. This latter limitation also applies to microsphere technique in addition to the problem of lack of flow monitoring within small volume of tissues in both techniques(e.g. a few cubic millimeters). Other disadvantages of the microsphere technique are the use of radio-isotopes involvement and the need to sacrifice the animal or remove the piece of tissue under study which limits its clinical usefulness (Tothill 1984). Transient changes in blood flow occurring over a few seconds may not be detected because flow is not measured continuously, and the number of measurements of flow per animal is limited. Meanwhile, radio-labelled microsphere method offers several distinct advantages for measuring blood flow: it measures tissue blood flow quantitatively, total and regional blood flow can be determined, and experiments can be done using both anaesthetised and conscious animals.

On the other hand some sources of error in measuring regional blood flow with this technique were reviewed by Buckberg and his colleagues (1971). They concluded that using small numbers of microspheres, inadequate mixing of them before injection, disposition of the tip of catheter in the heart, and the larger the size of microsphere could all be sources of error in blood flow measurements. However, in present study attempts were made to avoid or

minimize these errors by using enough microspheres ($1X10^6$-$2X10^6$). According to a recent study, it was concluded that $0.5X10^6$ spheres per kg weight would be enough for an accurate estimation of blood flow in tissue such as bones (Li et al 1989), so it seems this number of spheres was adequate to have enough number of radio-labelled isotopes in bones and muscles but in posterior and anterior capsules the problem of inadequate number of spheres was addressed by increasing the number of experiments, and the results then became significant. The 15um size of microspheres were chosen as in a report by Tothill(1984) it was found that the most accurate measurement of blood flow was obtained using 15um microspheres. This was also confirmed by others (Morris and Kelly 1980, Li et al 1989). Checking that the tip of the cannula was in the left ventricle was carried out by monitoring ventricular pressure during the experiment except during the injection phase, and also at the end of experiment by opening the heart. And finally the adequate mixing of microspheres was done by shaking the solution containing microsphere thoroughly with whirlimixer before injection, and it was checked by examining both kidney counts.

A more recent technique which has been used to estimate blood flow changes in different organs and tissues is the Laser Doppler Flowmetry (LDF) technique (Holloway and Watkins 1977, Stern et al 1979, Ahn et al 1985, Haberl et al 1989a,b). The ability of LDF to analyze

a very limited tissue volume, and provide instantaneous and continuous measurement of tissue blood flow in recent experiments proved to be of great value. Other advantages of this technique are that it is easy to transport and to handle, and being non-invasive and is applicable to man in some aspects. However, a limitation of the Doppler method is that changes in blood velocity differ from changes in blood flow when vessel diameter changes (Busiji et al 1981). Another limitation of this method is that LDF monitors relative changes in blood flow rather than absolute change. Positioning of the fibre optic probe in proximity to the joint capsule was the most difficult aspect of this study, and sometime, several different approaches for obtaining a proper probe position were tried. When an appropriate and constant flux signal was achieved, the probe was firmly fixed in position. An indicator of a clear flow signal was the pulsatile variation of flow when averaged with a 0.5sec time constant.

LDF appears to be a sensitive and reliable technique for real-time assessment of relative changes of blood flow in the synovium and periarticular structures of the knee joint. The response time (<0.5 sec) and sensitivity of the flowmeter allowed recording of pulsatile variations of microvascular flow as well as changes in flow due to nerve stimulation. In summary, LDF is easy to used and appears to be a reproducible technique for evaluating joint blood flow, offering distinct advantages over the microsphere technique for measuring joint blood flow.

Microspheres

The radio-labelled microsphere method has been used for circulatory studies since the late 1940s. The history of its development has been reviewed by Wagner et al (1969) and Heyman et al (1977). Recently this technique has been used extensively as an accepted method in different studies to evaluate the LDF technique (Busija et al 1981, Kviety et al 1985, Notzi et al 1989). Busija and colleagues (1981) concluded that LDF and microsphere methods, which are based on different principles, yield similar values for changes in cerebral blood flow from control. In another study by Kviety and his colleagues (1985), they suggested that, although the LDF and microsphere estimates of blood flow could not be expressed in the same unit (i.e. Volts vs ml/min/100g), the LDF signal from the mucosal surface was equally well correlated in a linear fashion, with microsphere estimates. This correlation between two techniques has been recently reviewed by Notzi and his colleagues (1989) in bone blood flow.

In present study on articular blood vessels, electrical stimulation of the nerve supply to the knee joint(20Volt amplitude, 1msec pulse width, 30HZ, and train of 1 minute duration) reduced capsular blood flow in both species of animals. The percentage changes in the laser flow meter signal due to nerve stimulation being significantly correlated with the percentage changes in

blood flow as measured with the microspheres ($r=0.908$; $p<0.001$; $n=17$). In some experiments a correlation has been shown between percentage change in the arterial blood pressure and laser flow meter signal ($r=0.992$, $P< 0.001$, $n=8$). The close correlation between the two methods additionally supports the evaluation of laser Doppler as a useful instrument. These results also strongly indicate that LDF has potential applications not shared by other techniques for assessing articular blood flow. Among these are the instantaneous and continuous recording of changes in knee joint blood flow. This technique has very recently been used in the human knee joint to measure synovial blood flow changes (Geborek et al 1989), and the results also suggest that, since the laser light does not damage the tissue and no invasive preparation other than inserting a 18G-needle is necessary, LDF may aid in assessing capsular blood flow in the human knee joint.

Nerve stimulation

Electrical stimulation of posterior articular nerve in the cat, not only reduced capsular blood flow in both animals but also decreased the blood flow to tibia and femur as well. These reductions in flow of tibia and femur due to stimulation of saphenous nerve in the rabbit were less compared to the cat. These results indicate that in the cat and in the rabbit , blood vessels in tibia and femur are innervated by presumed sympathetic vasoconstrictor fibres travelling in nerves supplying the

joint. However, the mean blood flow of the tibia and femur were not significantly different between two legs in both species of animals after denervation of the experimental limb. The explanation for this might be less sympathetic tone in the nerves supplying the blood vessels in femur and tibia. Another aspect of these results concerns the values of blood flow to these tissues. As shown in table 5 and 6, the blood flow of the experimental and control legs in the femur and the tibia were not significantly different. These result are consistent with the finding of other investigators (Morris and Kelly 1980, Li et al 1989). The blood flow of the femur measured in the rabbit is comparable to the finding of Morris and Kelly (1980) in the dog. But the mean values for tibia are different from their finding. By comparing two species, although the blood flow in the rabbit capsular regions are greater compare to the cat, but there is not much differences in the blood flow of the tibia and the femur between two species.

The values of flow in control legs for the capsular region in the cat are comparable to that obtained by Bunger et al (1983) in the dog knee (1.1 ± 0.5ml/min/100g). It was noticeable that blood flow in anterior joint capsules after the second injection prior to nerve stimulation in both species was always greater in the experimental leg than the control leg. Comparing the results after first and second injections of different microspheres, the increase in blood flow of posterior

capsule in the experimental leg is most likely be due to the loss of sympathetic tone after denervation of this limb. But, since the probe was inserted from anterior aspect into the joint cavity in both species, and there is significant rise in blood flow of anterior capsules after this, this increase in flow is probably due to injury hyperaemia.

Application of The LDF technique

This new method for measuring articular blood flow can have wide applications in the study of these vessels within normal and diseased knee joints. Articular blood flow can be measures virtually continuously. Thus transient and steady-state responses of the joint blood flow to physiological stimuli and drugs can be observed and as percentage change quantified. In addition, when changes in blood flow measurements during nerve stimulation or effect of drugs injection are needed, this method can serve as a guide for the timing of injection of microspheres.

****

****

****

****

***

*

CHAPTER FOUR

THE INNERVATION OF ARTICULAR BLOOD VESSELS IN THE RABBIT

SECTION I

RECEPTORS ON THE KNEE JOINT BLOOD VESSELS IN THE RABBIT

A. Adrenoceptor profile

SUMMARY

1. In vitro and in-vivo experiments were carried out on the rabbit knee joint to assess the nature of adrenoceptors within articular blood vessels.

2. Dose/response relationships were obtained to intra-arterial injection of α and β adrenoceptor agonists.

3. Adrenaline and noradrenaline produced a similar pattern of increasing constriction of articular vessels with increasing dose of drug.

4. The α_1 agonist, phenylephrine, also produced dose dependant constrictor responses, but the α_2 agonist, clonidine, had no effect. The α_2 agonist UK-14304 did, however, produce constriction which became more obvious when the tone of the blood vessels was raised. These responses were not greatly altered by prazosin. The constrictor effect of noradrenaline was abolished by phenoxybenzamine and prazosin but not by the α_2 blocker rauwolscine.

5. The β adrenoceptor agonist, isoprenaline had little effect at a dose of 10^{-6}M or lower, but gave rise

to a constrictor effect at higher concentrations. This response was blocked by phenoxybenzamine but not by propanolol, suggesting that this constrictor effect was mediated via α adrenoceptors.

6. When constrictor tone was enhanced by perfusion with vasopressin, no significant dilatation was observed upon injection of isoprenaline in doses up to 10^{-4}M.

7. The results suggest that α_1 and α_2 adrenoceptors are present within articular blood vessels, but that β receptors are absent. The effects of noradrenaline appear to be mediated principally via α_1 adrenoceptors.

INTRODUCTION

The maintenance of a stable intra-articular environment is critically dependent on synovial fluid formation and the factors determining this have been described in detail by Levick (1984). The perfusion pressure across the synovial vascular bed is known to be an important determinant of trans-synovial flow (Knight & Levick 1984). However, relatively little is known about those factors influencing articular blood vessel calibre. It has been shown that blood vessels in the dog knee joint are innervated by sympathetic efferent nerve fibres whose action is to constrict these vessels (Cobbold & Lewis 1956a). This finding was confirmed in a more recent study on the cat knee joint (Ferrell & Cant 1987).

As to the types of adrenoceptors present on articular blood vessels, rather less is known. Cobbold & Lewis (1956b) found that close intra-arterial injection of adrenaline and noradrenaline both produced vasoconstriction, although noradrenaline produced consistently greater responses. Although not commented upon, this finding could be explained by the presence of ß receptors on these blood vessels in addition to α receptors. Another study by Dick and his colleagues (1971), indicated that α and ß adrenoceptors were functionally active in the control of synovial perfusion in normal human subjects. However, these authors do not appear to have systematically investigated the

dose/response relationship of these agents, and were therefore unable to establish their relative potencies. The object of this section was to perform a more extensive and quantitative investigation using selective α and β receptor agonists and antagonists in order to characterise the types of adrenoceptors present on knee joint blood vessels in the rabbit.

EXPERIMENTAL PROTOCOL

a:In-vitro

Experiments were performed on albino New Zealand rabbits of either sex, which were killed by a blow to the skull and exsanguinated. The isolated knee joint preparation was then transferred to a thermostatically controlled bath as described previously (Chapter 2,II,a). In the experiments on the completely isolated capsule, after the cannulation of the popliteal artery, the posterior capsule along with the cannula was removed by cauterising the edge of capsule to seal the blood vessels. The perfusion rate needed to keep same perfusion pressure (40-50mmHg) in these experiments was lower (0.1-0.2 ml/min) than the in-situ preparation. It was noticeable that in one of the experiments, the rechargable batteries of the cautery device discharged before completion of the cauterisation, so the rest of procedures (cutting the edge of capsule) were carried out by using scissors. As a result, the same perfusion rate (0.1-0.2ml/min) only produced a perfusion pressure of 15mmHg. This is probably explained by the lower resistance of the transected vessels. Therefore in the in-vitro preparation when the capsule was in place (in-situ), a higher flow rate was required because of those surrounding vessels which anastomose with ones supplying the joint, were transected during removal of muscles and tendons. Changes in

perfusion pressure thereafter provided an indirect measure of articular blood vessel calibre. Peak systolic response was compared to control (pre-injection) value and expressed as percentage change from control (or baseline). "Control" injections of Locke's solution were administered periodically and were found to produce little change in pressure, apart from a transient rise during the injection phase.

b: In-vivo

Experiment were carried out in New Zealand rabbits of either sex weighting approximately 1.8 to 3.0 Kg. They were anaesthetized initially with hypnorm (0.1mg/kg, im) and diazepam (0.5mg/kg, ip) and this anaesthesia was maintained thereafter by using a halothane (1-2%), N_2O/O_2 which was delivered via a tracheal cannula. The trachea was intubated for this purpose. A cannula was inserted into the left carotid artery for the measurement of blood pressure. Arterial blood pressure was recorded by a physiological pressure transducer (Elcomatic EM 750). The cannula was flushed periodically with heparinized saline to prevent clotting.

The saphenous artery was cannulated for either injection of drugs (agonists and antagonists) or slow infusion of some antagonist(e.g. guanethidine).

Relative changes in articular blood flow was

monitored as described in chapter two (II,b,2,iv).

RESULTS

a: The effect of Adrenaline and Noradrenaline

Intra-arterial injection of adrenaline and noradrenaline resulted in a dose dependent vasoconstriction of knee joint blood vessels both in-vivo and in-vitro(Figures 4.1A,4.2, 4.3). Although in in-vitro preparations the dose/response profiles differ at 10^{-6}M and 10^{-3}M, these show considerable overlap elsewhere. For both agents the response at each dose differed significantly from the response evoked by the preceding dose except for the highest dose of noradrenaline and the 10^{-5}M dose of adrenaline. The response to adrenaline was also tried in a total isolated knee joint capsule. As the results show (Figure 4.1A), there is no significant difference between these responses compared to dose-response curve of adrenaline in same vessels when the capsule was in-situ. These results suggest that there is little difference between the study of capsular blood vessels in the completely isolated capsule or in the in-situ preparation. But since it was shown that only articular blood vessels were perfused by Locke's solution in the in-situ preparation (Figure 2.16), this was utilised because 1) it is more physiological than the isolated preparation. 2) In the field stimulation experiments, it was easier to handle and locate the electrodes with the capsule in situ. The responses to

Fig. 4.1. A: Change in perfusion pressure (mean ± SEM) with increasing doses of noradrenaline (□),and adrenaline in situ (■). Dose response curve for adrenaline in completely isolated capsule is also shown (O). n = 6 - 10.

B: Response to increasing doses of noradrenaline before(■)and during perfusion with 10^{-5}M rauwolscine( ) 10^{-6}M prazosin (Δ), and 10^{-5}M prazosin (O). n = 6 - 11 * $p<0.05$; *** $p<0.001$.

The concentrations can be converted to the mass of drug injected by multiplication of that concentration by $2x10^{-4}$ in this and subsequent figures.

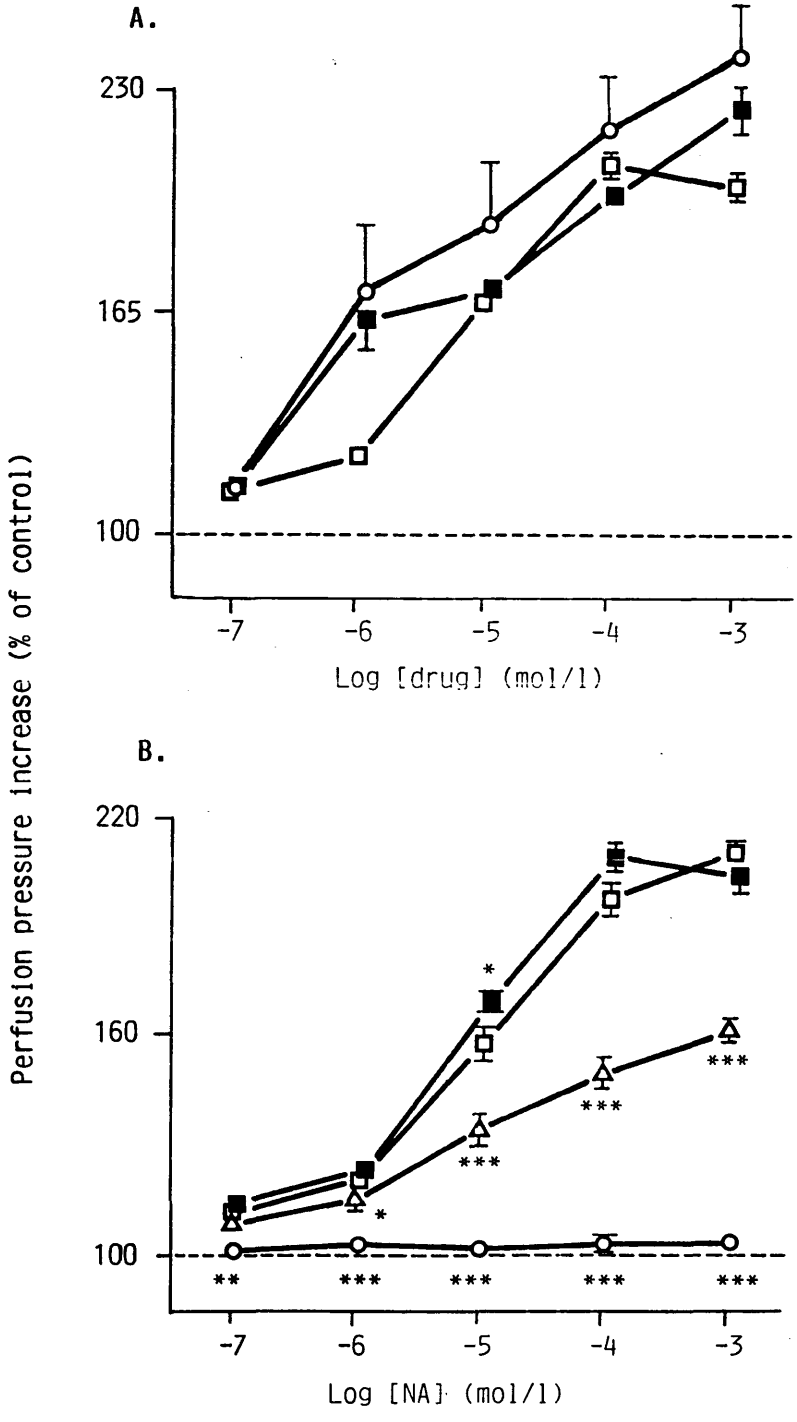

A.
230
165
100
-7
-6
-5
-4
-3
Log [drug] (mol/l)
B.
220
160
100
Perfusion pressure increase (% of control)
*
***
***
***
*
**
***
***
***
***
-7
-6
-5
-4
-3
Log [NA] (mol/l)

Fig. 4.2. Traces showing dose-dependent constrictor effects of noradrenaline injection on the perfusion pressure before (upper and middle) and after injection α-blocker, phenoxybenzamine, 10^{-3}M (lower).

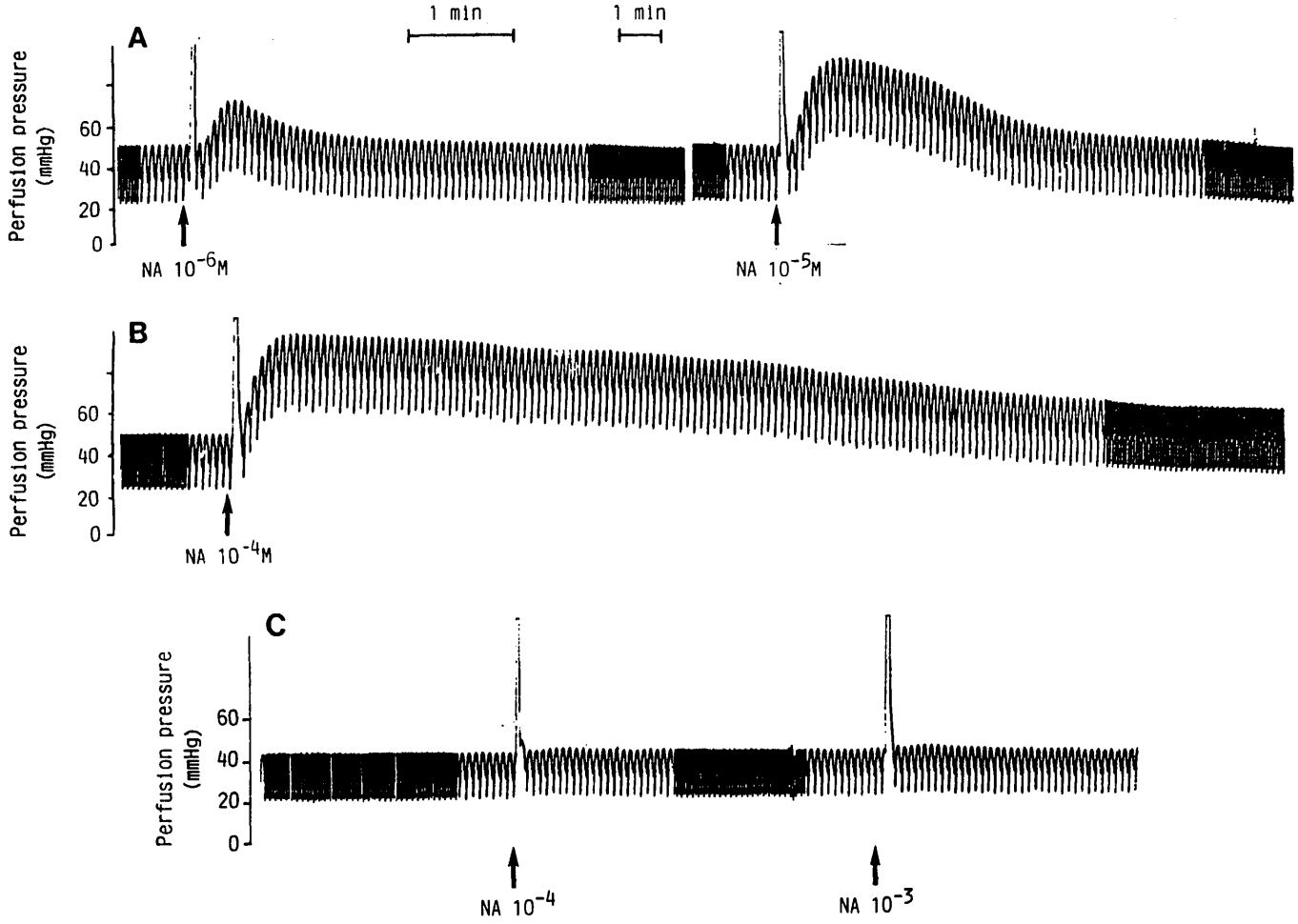
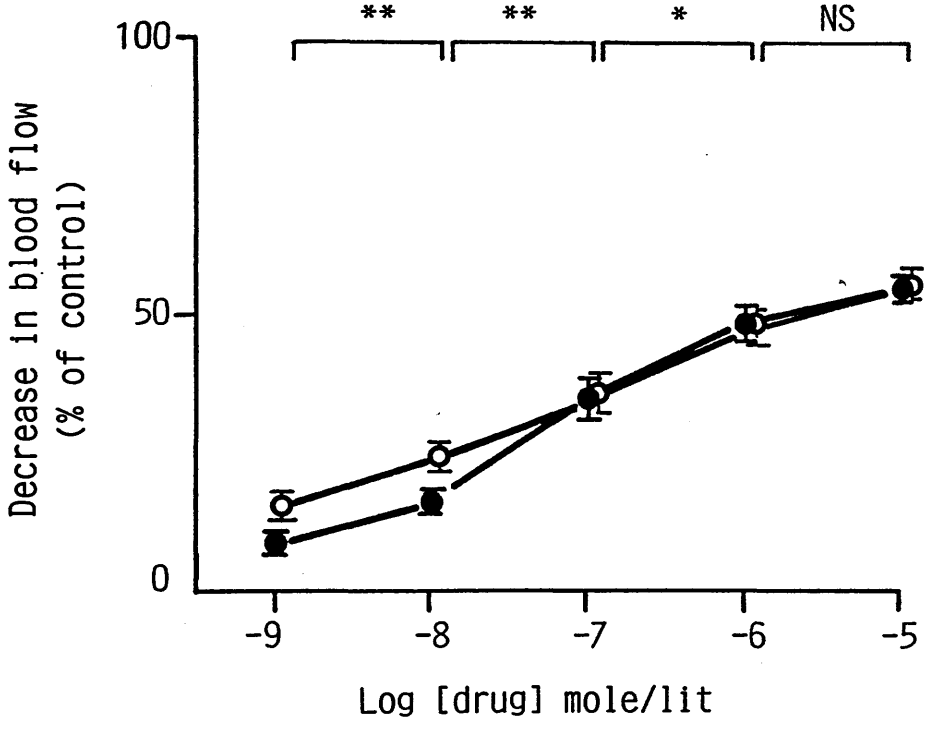

A
1 min
1 min
Perfusion pressure (mmHg)
60
40
20
0
NA 10-6M
NA 10-5M
B
Perfusion pressure (mmHg)
60
40
20
0
NA 10-4M
C
Perfusion pressure (mmHg)
60
40
20
0
NA 10-4
NA 10-3

Fig. 4.3. Change in blood flow (mean ± SEM) with increasing doses of adrenaline(O) and noradrenaline(●) in-vivo, n= 5 - 8, NS; Non significant, * P<0.05. ** P<0.01.

Stars show the significant differences between doses drug.


**
**
*
NS
100
50
0
Decrease in blood flow
(% of control)
-9
-8
-7
-6
-5
Log [drug] mole/lit

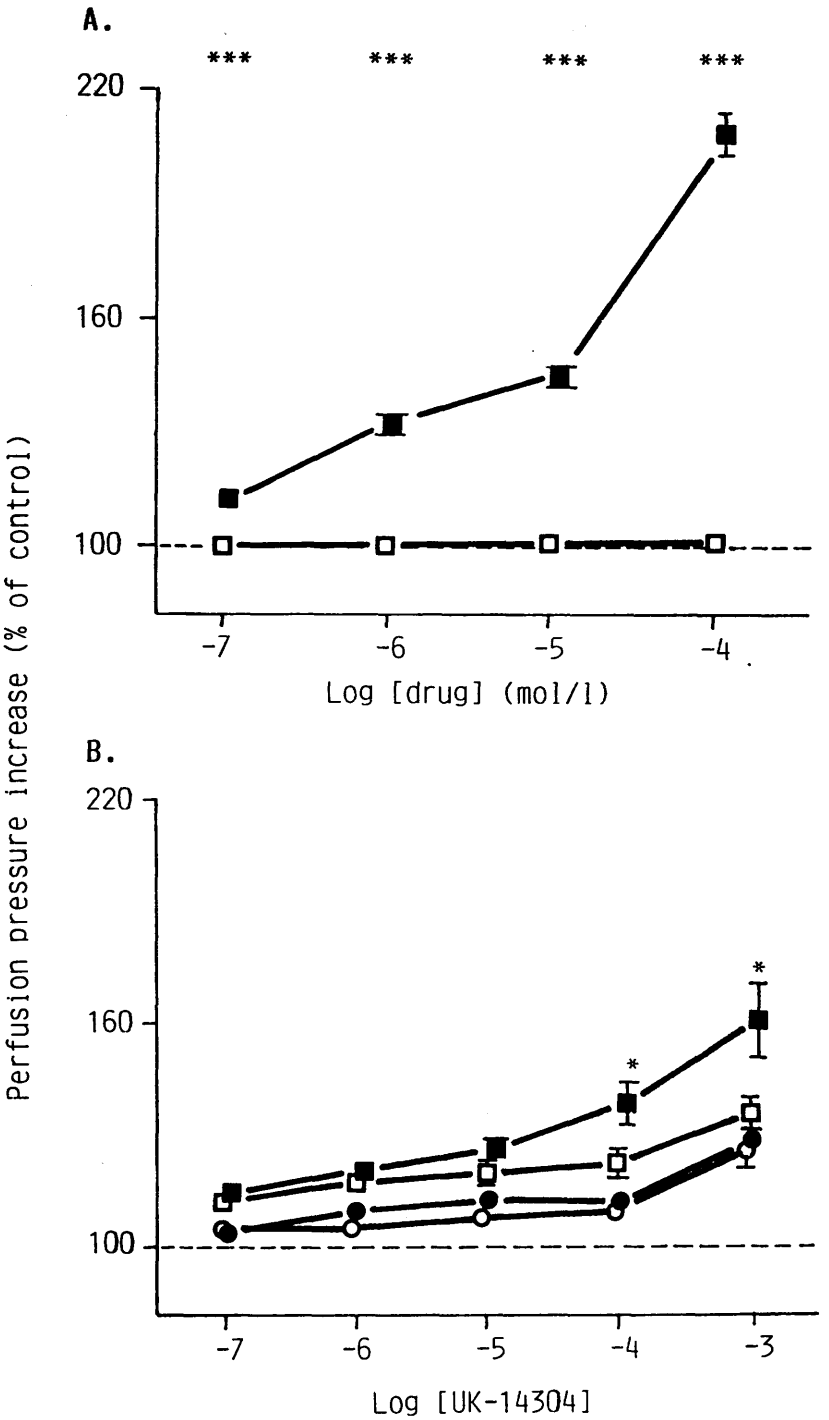
adrenaline and noradrenaline were found significant in about 10^{-9}M in-vivo whereas response became significant when 10^{-7}M concentration of these drugs were injected in-vitro.

b: The effect of Phenylephrine, Clonidine, and UK-14304

To further investigate the nature of adrenoceptors on articular blood vessels, α_1 and α_2 agonists were administered in the in-vitro preparation. Comparison of the effects of the selective α_1 agonist phenylephrine with the selective α_2 agonist clonidine (Figure 4.4A) reveals the contrast between the powerful constrictor effect of phenylephrine, which compares favourably with the effects of adrenaline and noradrenaline, and the lack of response to clonidine. However, when another selective α_2 agonist (UK-14304) was used, it was found that constrictor responses were obtained at higher doses and these were unaffected by prazosin (Figure 4.4B), suggesting that this effect was mediated by α_2 adrenoceptors. Raising the tone of blood vessels by the addition of angiotensin II (5×10^{-8}M) enhanced the response to UK-14304. Under these conditions prazosin had little effect at low doses of UK-14304, suggesting that these responses were mediated by α_2 adrenoceptors. However, at higher doses (10^{-4} and 10^{-3}M) prazosin produced significant reduction of the constrictor response to UK-14304, suggesting involvement of α_1 adrenoceptors as well. In-vitro, the effect of

Fig. 4.4. A: Responses (mean ± SEM) to α_1 agonist phenylephrine (■),and α_2 agonist clonidine(□). n = 6-12. *** $p<0.001$.

B: Responses to α_2 agonist UK-14304 before (●) and during perfusion with 10^{-6}M prazosin (O). n = 6 - 13. Upon raising the tone of the blood vessels by perfusion with $5x10^{-8}$M angiotensin II, the response to UK-14304 (■) is enhanced. Addition of 10^{-6}M prazosin to the perfusate reduces the responses at higher doses(□). n= 6-13. * $P<0.05$


A.
***
***
***
***
220
160
100
-7
-6
-5
-4
Log [drug] (mol/l)
B.
220
160
100
*
*
-7
-6
-5
-4
-3
Log [UK-14304]
Perfusion pressure increase (% of control)

UK-14304 was tested in eleven preparations and produced responses in nine of these. In two cases no responses were observed even during angiotensin II infusion.

The effect of UK-14304 was also tested in-vivo and the results show a dose dependent vasoconstriction of articular blood vessels in all animals.(Figure 4.5), but with greater sensitivity compared to the response observed in-vitro.

c: The effect of Prazosin and Rauwolscine

The nature of the adrenoceptors mediating the reponse to noradrenaline was investigated by using selective α adrenoceptor blockers in the in-vitro preparation. Figure 4.1B shows that the response to increasing doses of noradrenaline was reduced by 10^{-6}M prazosin and abolished by 10^{-5}M prazosin, whereas the α_2 blocker rauwolscine had little effect. These results suggest that the constrictor effect of noradrenaline was mediated via α_1 adrenoceptors.

d: The effect of Isoprenaline

The presence of ß adrenergic receptors was investigated in-vitro, by administration of the ß agonist isoprenaline. As illustrated in Figures 4.6, 4.7A, there was little effect up to a dose of 10^{-6}M, but thereafter a significant constrictor response was obtained. The

Fig. 4.5. Responses of articular blood vessels in the rabbit knee (mean ± SEM) to close intra-arterial injection of increasing doses of UK-14304, an α_2 agonist. In-vivo preparation. n= 8-12

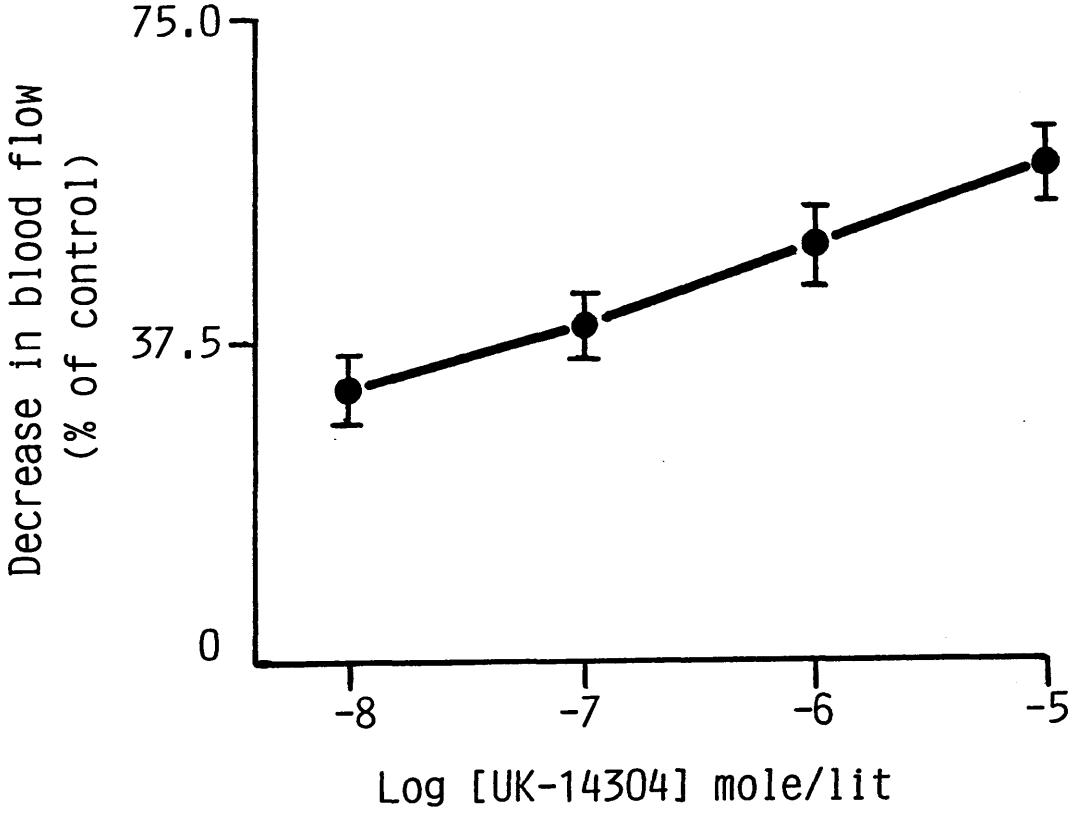

75.0
37.5
0
Decrease in blood flow
(% of control)
-8
-7
-6
-5
Log [UK-14304] mole/lit

Fig. 4.6. Trace showing the rise in articular vascular tone by perfusing with vasopressin 10^{-8}M (A),and the effect of Locke solution , and isoprenaline injections (ISO).

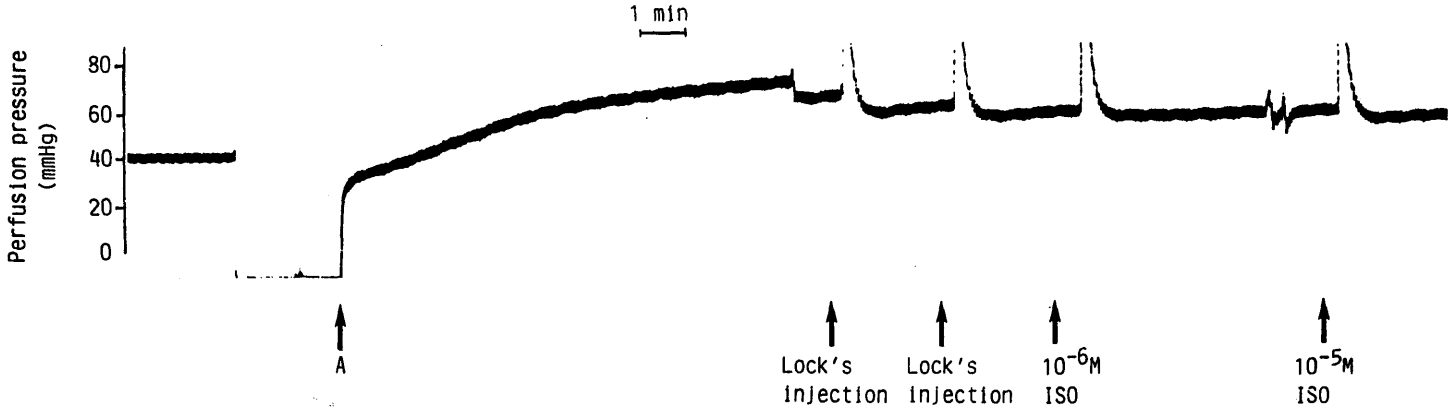
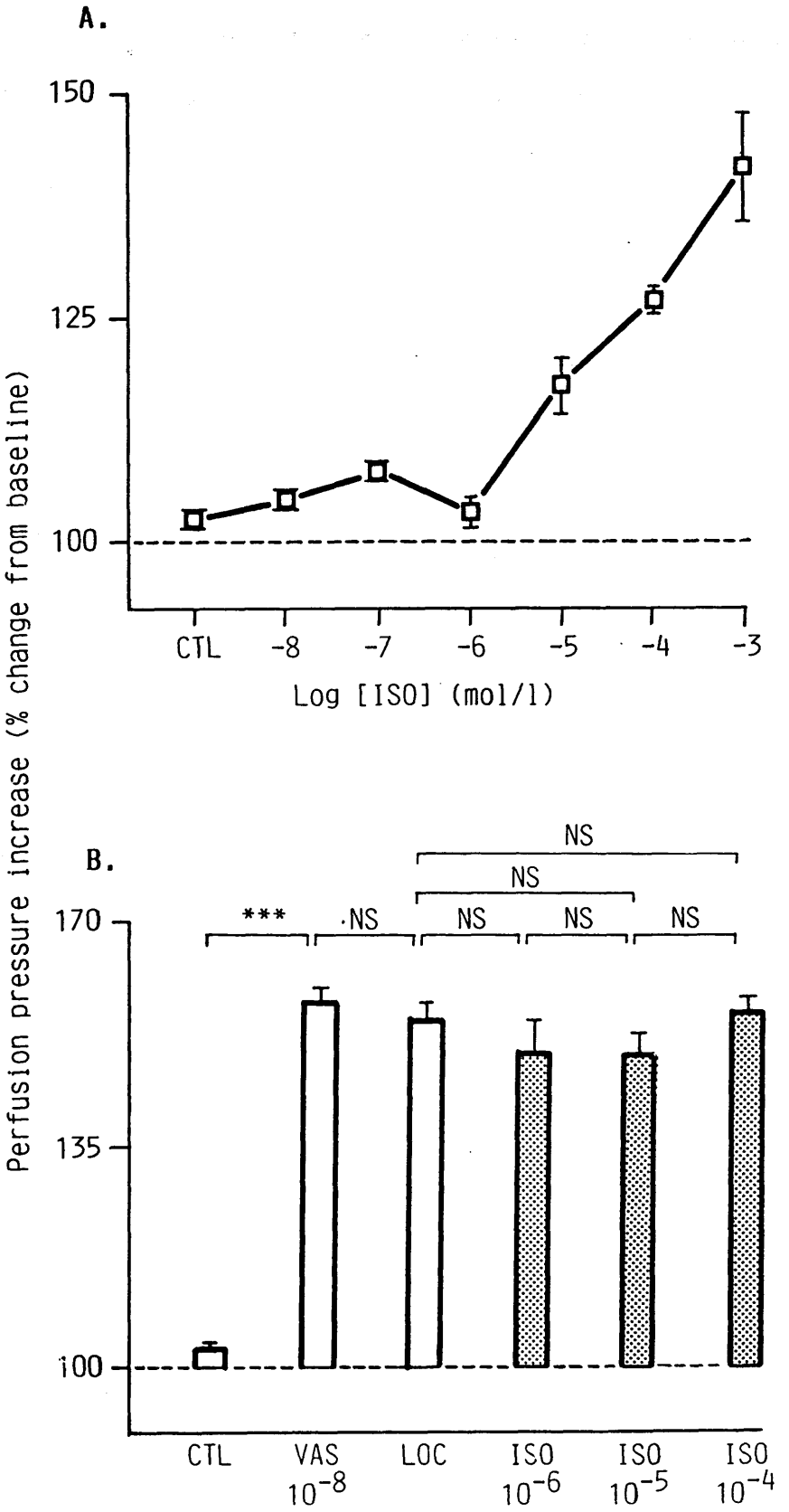

1 min
Perfusion pressure (mmHg)
80
60
40
20
0
A
Lock's injection
Lock's injection
10^{-6}M ISO
10^{-5}M ISO

Fig. 4.7. A: Responses (mean ± SEM) to increasing doses of isoproterenol (ISO). CTL is the response to injection of Locke's solution. Values are compared to the baseline value prior to injection of any agents. n= 6-8. * $P<0.05$, ** $P<0.01$.

B: Responses (mean ± SEM) to three doses of isoproterenol (shaded histogram) whilst blood vessel tone was elevated by perfusion with 10^{-8}M vasopressin (VAS). LOC is the response to injection of Locke's solution(blank histogram). No significant vasodilatation occurs under these conditions.NS; values not significant, *** $P<0.001$.

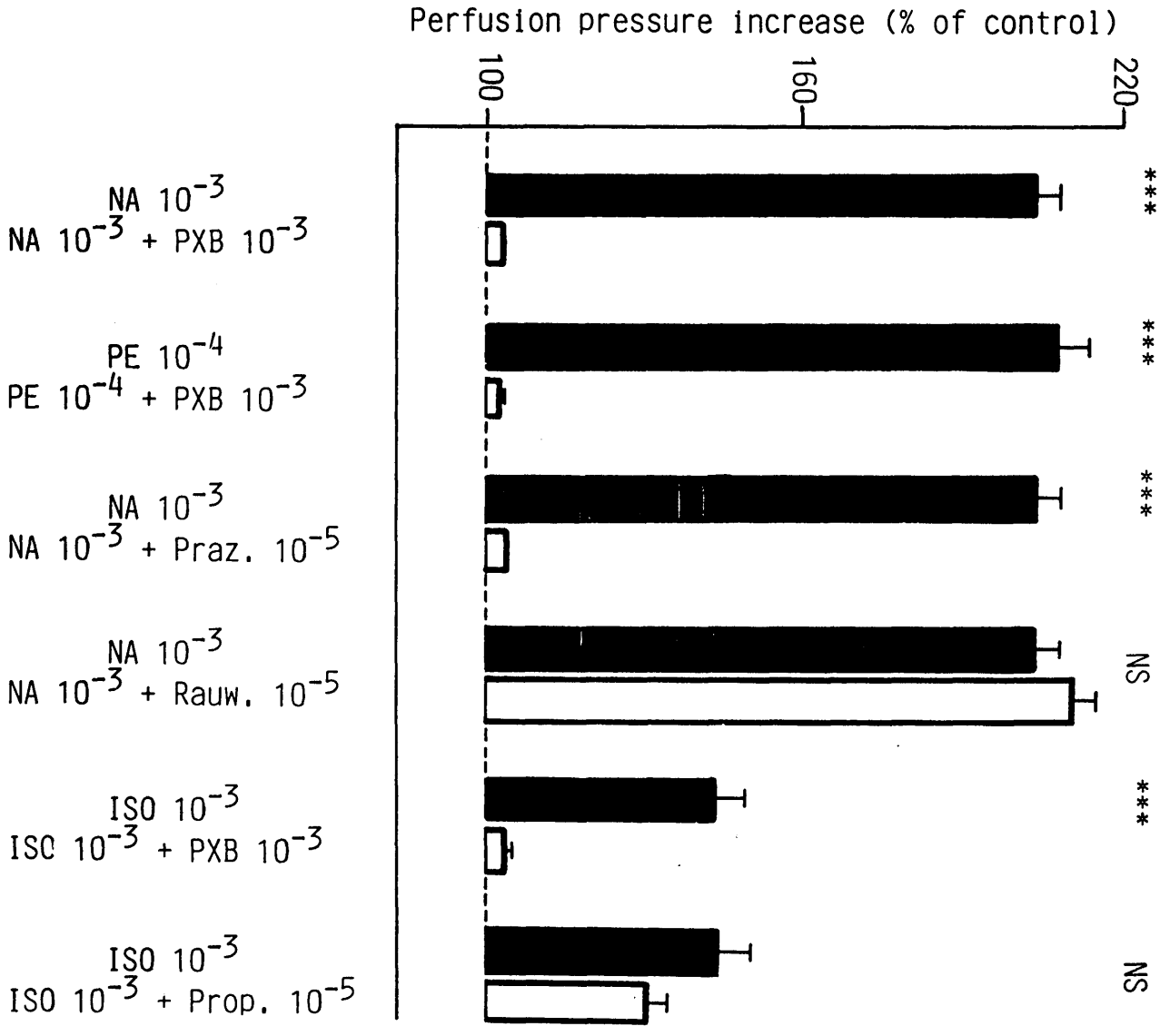

A.
150
125
100
CTL
-8
-7
-6
-5
-4
-3
Log [ISO] (mol/l)
Perfusion pressure increase (% change from baseline)
B.
NS
NS
***
NS
NS
NS
NS
170
135
100
CTL
VAS
10^{-8}
LOC
ISO
10^{-6}
ISO
10^{-5}
ISO
10^{-4}

inability to detect any dilator response could be attributed to a low inherent basal constrictor tone in the absence of tonic sympathetic outflow. Thus, constrictor tone was enhanced by perfusion of the tissue with vasopressin (10^{-8}M), and isoprenaline again administered in increasing doses. However, no significant dilator effect was observed even at high doses (Figure 4.7B).

e: The effect of Phenoxybenzamine and Propranolol

The constrictor effect of isoprenaline illustrated in Figure 4.6 was mediated via α adrenoceptors as it could not be blocked by propanolol (10^{-5}M) but was substantially reduced by phenoxybenzamine (10^{-3}M), as shown in figure 4.8. Also it has been shown that the constrictor effects of phenylephrine and noradrenaline are blocked by phenoxybenzamine, with only rauwolscine having no antagonistic effect on the actions of noradrenaline.

Fig. 4.8. Comparison of the the effects of phenoxybenzamine (PXB) on noradrenaline (NA), phenylephrine (PE) and isoproterenol (ISO). PXB blocks the response to NA and PE. The constrictor effect of ISO is not blocked by propanolol but by PXB. The response to NA is blocked by prazosin but not by rauwolscine. n=6-9, means $\pm$ SEM, NS; Non significant, * $P<0.05$, *** $P<0.001$.


Perfusion pressure increase (% of control)
100
160
220
NA 10^{-3}
NA 10^{-3} + PXB 10^{-3}
PE 10^{-4}
PE 10^{-4} + PXB 10^{-3}
NA 10^{-3}
NA 10^{-3} + Praz. 10^{-5}
NA 10^{-3}
NA 10^{-3} + Rauw. 10^{-5}
ISO 10^{-3}
ISO 10^{-3} + PXB 10^{-3}
ISO 10^{-3}
ISO 10^{-3} + Prop. 10^{-5}
***
***
***
NS
***
NS

DISCUSSION

The results of the present experiments clearly demonstrate the presence of α_1 adrenoceptors on articular blood vessels. There was little systematic difference in the constrictor response to adrenaline and noradrenaline across a range of doses in-vitro and just at very low doses (10^{-9}M) in-vivo, suggesting that these agents were acting solely on α adrenoceptors. In low doses the constrictor effect of adrenaline in-vivo was found to be greater than that of noradrenaline. This could be the result of β adrenoceptor activation leading to vasodilation in striated muscles around the knee joint (Berne and Levy 1985) which shunted blood flow into these areas and thus away from articular blood vessels. This differs from the findings of Cobbold & Lewis (1956b) who observed that noradrenaline produced more powerful constrictor effects on dog knee joint blood vessels. However, as they did not quantitate their findings, it is difficult to assess the significance of this effect. Another explanation for this discrepancy may reside in species differences.

Since the arterial branches close to knee joint blood vessels were left unligated in the in-vivo experiments, some of injected drugs can enter these branches and thus less of the drug would reach to articular blood vessels. Even in this condition, there were greater responses to

intra-arterial injection UK-14304 in-vivo compare to the in-vitro effects. The explanation for this might be synergic interaction of UK-14304 with circulating substances such as vasopressin and angiotensin II in the in-vivo preparation. This is supported to some extent by the observation that the response to UK-14304 was greater in-vitro when vascular tone was elevated by angiotensin II (Figure 4.4B). Another explanation could be that postsynaptic α_2 -adrenoceptors are more sensitive to UK-14304 in-vivo.

Although Dick et al (1971) found that in human articular blood vessels both α and β adrenergic receptors were present, this does not apply in rabbit knee joint vessels where β adrenoceptors are absent. The constrictor effect of isoprenaline as mediated by α adrenoceptors as it could not be blocked by propanolol but was blocked by phenoxybenzamine. Even with enhanced constrictor tone induced by perfusion with vasopressin, elevated doses of isoprenaline failed to evoke significant vasodilatation. Therefore the results of present experiments conflict with those of Dick et al (1971). This could be due to differences in the methods employed or the species.

The results of these experiments suggest that the dose dependant constriction of articular blood vessels by noradrenaline is mediated principally via α_1 adrenoceptors. Although post-junctional α_2 adrenoceptors could be demonstrated, these would appear to contribute relatively little to constrictor responses <u>in vitro</u>.

An interesting feature of the present results is that although the popliteal artery divides to give muscular and articular branches in close proximity to each other, the adrenoceptor profile of articular blood vessels is closer to that of superficial tissues such as skin than that of blood vessels supplying muscle. Thus, although both skeletal muscle and joints are considered "deep" structures, there is not homogeneity in the distribution of adrenoceptor types which may reflect the differing function of these two vascular beds.

B. Purinergic, Histaminergic, and Cholinergic receptors.

SUMMARY

1. Experiments were performed in-vitro on the rabbit knee joint, to investigate the presence of purinoceptors, cholinergic receptors, and the role of endothelium within articular blood vessels.

2. The basal tone of the blood vessels was not affected by adenosine or acetylcholine. ATP injection produced vasoconstriction which was unaffected by removal of the endothelial layer, but diminished by α , β methylene ATP, a P_2-purinoceptor desensitiser.

3. When knee joint blood vessel tone was raised by perfusion with 10^{-8}M vasopressin or serotonin (10^{-5}M) , acetylcholine, ATP and adenosine were all found to induce concentration-dependent relaxation of these vessels. ATP was found to have a dual effect of transient constriction followed by longer-lasting dilation.

4. Methylxanthine, a potent P_1- purinoceptor antagonist significantly reduced the relaxation response to adenosine but had no effect on the vasodilator effect of ATP.

5. Removal of the endothelial layer virtually abolished the vasodilator effects of acetylcholine and ATP but not adenosine.

6. Intra-arterial injection of histamine induced vasoconstriction of articular blood vessels in-vivo.

7. These results demonstrate that articular blood vessels supplying the rabbit knee contain P_1-purinergic receptors located on the vascular smooth muscle which mediate vasodilation. P_2-purinergic receptors, mediating a constrictor effect are also present on this smooth muscle. It is likely that the vasodilator effect of ATP is mediated via P_2-purinoceptors located on the endothelial layer.

INTRODUCTION

In recent years attention has been focussed on purine nucleotides and nucleosides which have been found to have widespread and potent vascular actions (Haddy & Scott, 1968; Su, 1981; Burnstock, 1980, 1984). Some of these actions appear to be mediated via the endothelium as Furchgott (1981) found that removal of the endothelial layer of dog arteries abolished the vasodilator action of acetylcholine (Ach) ATP, ADP, substance P and bradykinin. Subsequently, it was shown that ATP, but not adenosine, induced vasodilation via a purinoceptor located on the endothelium in many different blood vessels (De Mey & Vanhoutte, 1981, 1983; Vanhoutte & Rimele, 1983; Burnstock & Kennedy, 1986). However, other studies have found that the vasodilator action of adenosine can be mediated via the endothelium (Gordan & Martin, 1983; Frank & Bevan, 1983). Burnstock & Kennedy (1985, 1986) have shown that ATP can produce vasoconstriction via P_2-purinoceptors located on vascular smooth muscle cells.

In this section the aim was to assess the types of purinoceptors present on articular blood vessels and whether or not any effects of ATP, adenosine and acetylcholine are mediated via the endothelium.

EXPERIMENTAL PROTOCOL

a: In-vitro

White New Zealand rabbits of either sex weighing between 2-2.3 kg, were killed by a blow to the skull followed by exsanguination. Immediately thereafter, the tissue preparation was carried out as described previously (Chapter two). The isolated knee joint preparation was then transferred to a thermostatically controlled bath (37± 1° C) containing oxygenated Locke's solution. After a 30min equilibration period a steady resting pressure was achieved and subsequent changes in perfusion pressure provided an indirect means of assessing articular blood vessels calibre. Other measurement were obtained as explained previously.

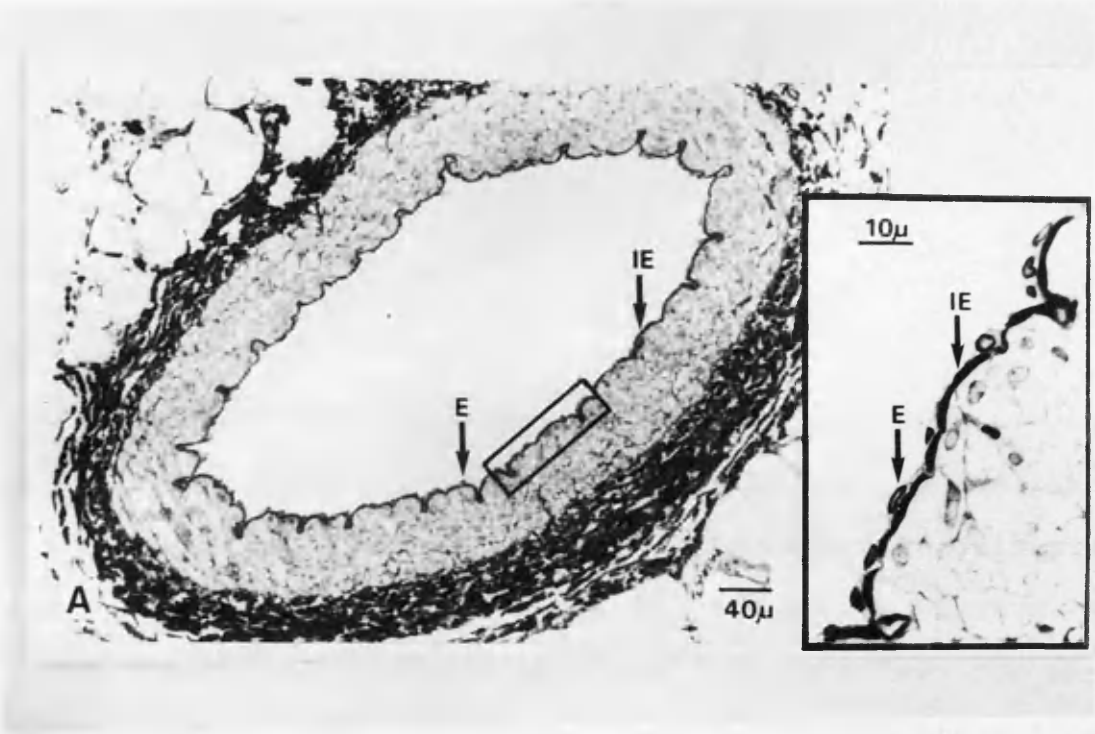
Vascular tone was increased by the addition of vasopressin 10^{-8}M or serotonin (5HT) 10^{-5}M or 10^{-6}M to the perfusate. These resulted in perfusion pressure increases of about 160-200% of control values.

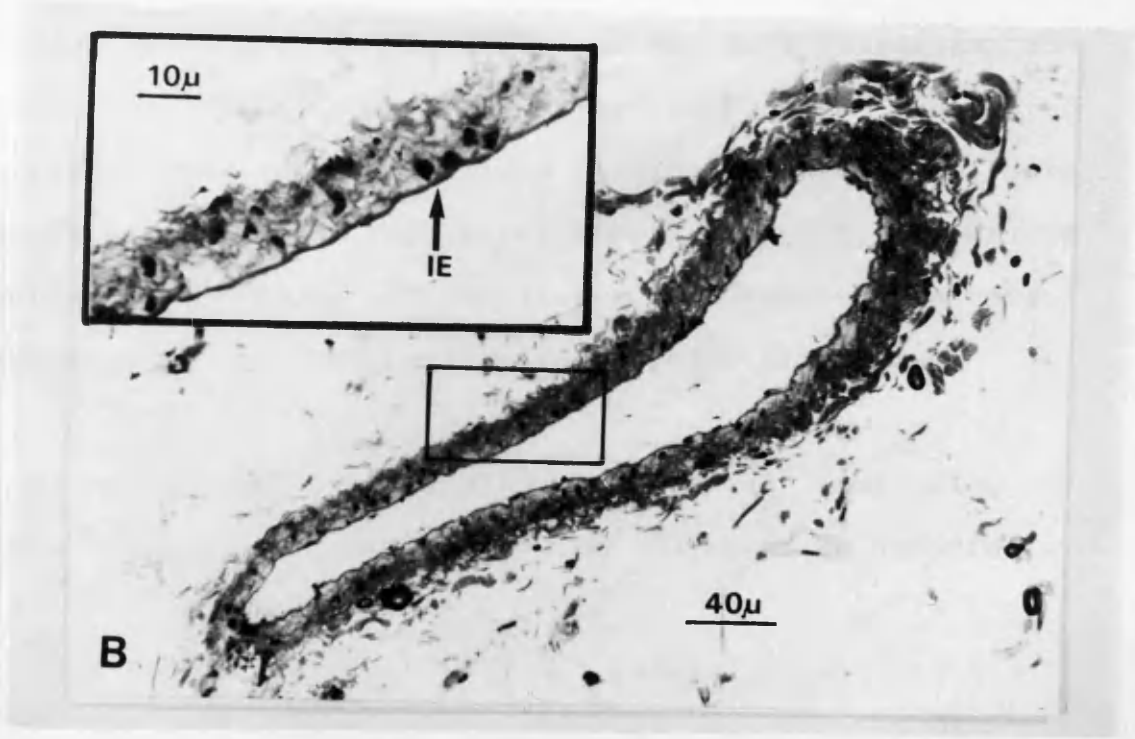
Removal of the endothelial layer in these preparations was achieved by perfusion with 1-3mg.ml^{-1} solution of sodium deoxycholate, in Locke's solution for 30sec on two separate occasions (Byfield, Swayne & Warner 1986). As illustrated in figure 4.9 , this procedure was effective in disrupting the endothelial layer five

Fig. 4.9. Photomicrographs of blood vessels from the rabbit knee joint.

A: Normal appearance of a muscular artery. Inset shows a higher power view (900X) of the area outlined, with endothelial cells (E) lining the internal elastic lamina (IE). Stained with Haemalun and Eosin (H & E).

B: After perfusion with sodium deoxycholate, endothelial cells no longer line the blood vessels. Inset shows the internal elastic lamina (IE) intact, but with absence of endothelial cells. Stained with Toludine blue.


IE
E
40μ
A
10μ
IE
E


10μ
IE
40μ
B

preparation tested.

b: In-vivo

White New Zealand rabbits weighting between 1.7 to 3.2 kg were anaesthetized with 0.1mg/kg hypnorm (im) and 0.5mg/kg diazepam (ip) injections. Thereafter anaesthesia was maintained by halothane (1-2%) in N_2O/O_2 mixture. A cannula was inserted into the right femoral artery for the measurement of blood pressure. To isolate the blood flow to the experimental limb, left femoral artery and right carotid arteries were cannulated. Then the blood from right carotid cannula was perfused to left femoral artery by a peristaltic pump. Thus changes in blood pressure had no further effect on blood flow of the left (experimental) leg. The left saphenous artery was also cannulated distally for drug injection. It is noticeable that there might be some other collateral supply to joint, but as the perfusion pressure was monitored, changes in arterial pressure did not obviously effect joint blood flow.

To prevent any clotting, at the beginning of experiment, each animal received 200 units/kg heparin.

RESULTS

a:Basal vascular tone in-vitro

(i). The effects of ATP and Adenosine

Intra-arterial injection of ATP(10^{-4}M to 10^{-2}M) produced vasoconstriction of articular blood vessels (Figure 4.10A) which was dose-dependent (Figure 4.10B). Maximum vasoconstriction was usually achieved within a minute, but was not maintained (Figure 4.10A). Almost no desensitisation was observed, even when the drugs were readministered after a short interval. Exogenous adenosine (10^{-7}M to 10^{-5}M) had no vasoconstrictor effect on knee joint blood vessels (Figures 4.10A,B; Table 7) but a slight and transient dilator effect was observed at 10^{-5}M. In a few preparations higher doses of adenosine were tried but no further vasodilation occurred.

(ii). The effect of Acetylcholine (Ach)

Acetylcholine in different doses (10^{-6}M to 10^{-4}M) failed to vasoconstrict articular blood vessels except in two preparations where Ach did elevate perfusion pressure when administered as a single dose.

TABLE 7

Table 7. Summary of the responses of rabbit knee joint blood vessels to injected ATP, adenosine, acetylcholine and α , β methylene ATP under control conditions, during perfusion of methylxanthine (10^{-5}M) and after removal of the endothelium by pretreatment with deoxycholic acid (DCA).

	Basal Tone		Elevated Tone		
Compound	control	DCA	Control	Methylxanthine	DCA
ATP	Contraction	Contraction [1]	Contraction then Relaxation	Contraction then Relaxation	Contraction only
Adenosine	slight relaxation $\geq 10^{-5}$M	___ [1]	Relaxation	NO Response	Relaxation [1]
Acetylcholine	Contraction [2] or relaxation [3] or no effect	___ [1]	Relaxation [2]	___	NO Response
α , β ,meATP	Contraction	Contraction [1]	Contraction	___	___

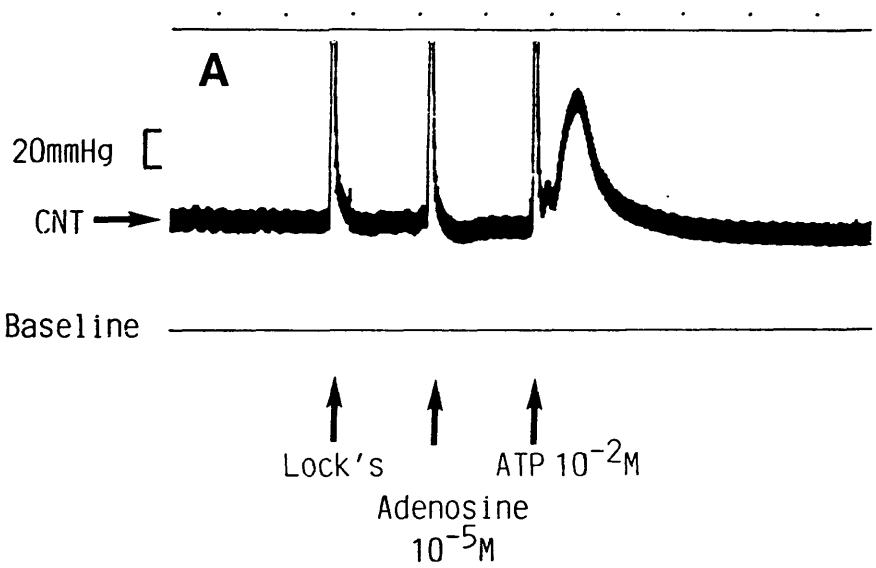
1. Experiments were carried out on a few preparations after pretreating the tissue with sodium deoxycholate but no significant changes occured compared to control response.
2. The response was antagonised by atropine 10^{-4}M.
3. Effects varied in different preparations.

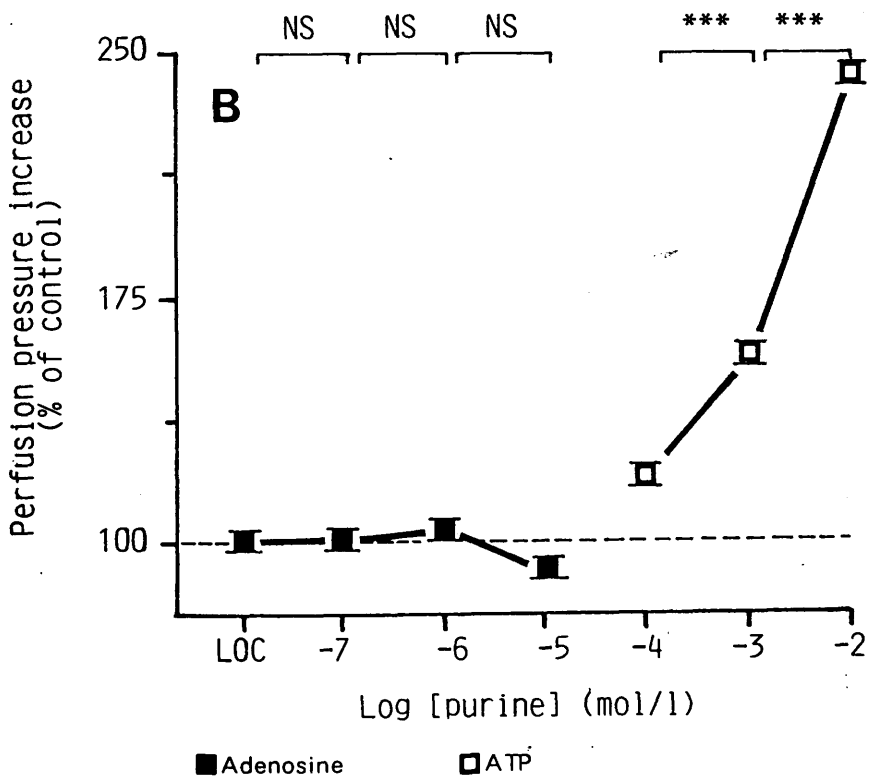
Fig. 4.10. A: trace showing the effect of injection of Locke solution, adenosine and ATP on the perfusion pressure. CNT indicates the control pressure prior to injection. The interval between each dot on the timescale indicates one minute.

B: Dose response relationship for adenosine (■) and ATP (□). LOC indicates response to injection of Locke solution. *** = $p<0.001$.

In both A and B the responses were obtained with basal vascular tone. For adenosine n = 7 at each dose, and for ATP n = 8 - 11.

The concentrations can be converted to the mass of drug injected by multiplication of that concentration by 2×10^{-4} in this and subsequent figures.


A
20mmHg
CNT
Baseline
Lock's
ATP 10^{-2}M
Adenosine
10^{-5}M


B
NS
NS
NS
***
***
250
175
100
Perfusion pressure increase
(% of control)
LOC
-7
-6
-5
-4
-3
-2
Log [purine] (mol/l)
Adenosine
ATP

(iii). The effect of α,β methylene ATP

Perfusion with α,β methylene ATP transiently increased the perfusion pressure but this was not sustained. None of the above constrictor responses was reduced by removal of endothelium (Table 7).

b.Raised vascular tone in-vitro

Vascular tone was raised by the addition of either vasopressin 10^{-8}M (before Ach injection) or serotonin (5HT) 10^{-5}M or 10^{-6}M, or histamine 10^{-6}M (before purine injections) to the perfusate.

(i). The effect of ATP and Adenosine

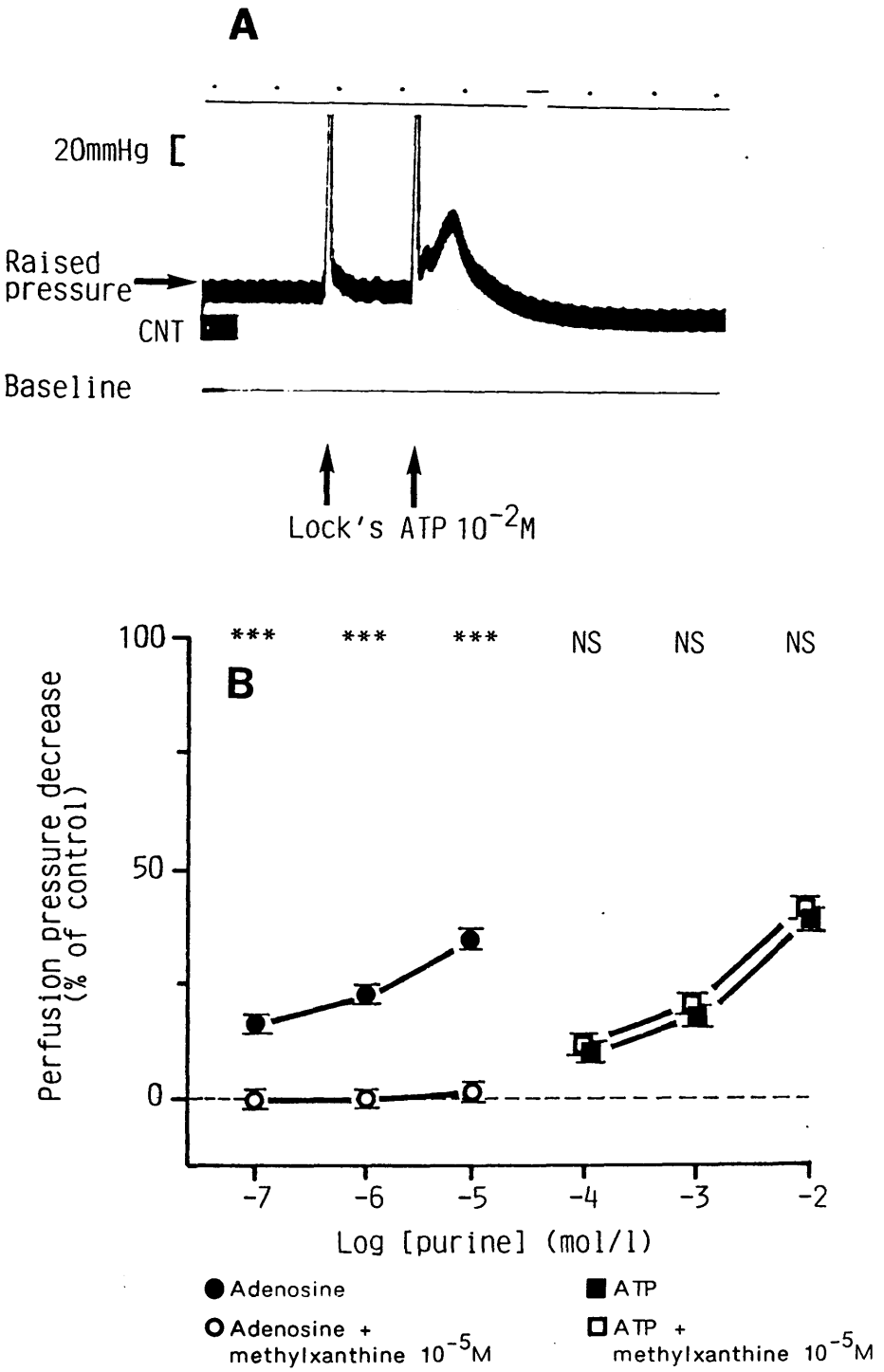
Adenosine (10^{-7}M to 10^{-5}M) and ATP (10^{-4}M to 10^{-2}M) were then found to produce concentration-dependent relaxation of articular blood vessels (Figure 4.11B) The dual effect of ATP is illustrated (Figure 4.11A), consisting of a transient constriction followed by a long-lasting dilatation. Only rarely was the initial constrictor response absent.

(ii). The effect of antagonists

Perfusion of the preparation with 3-methylxanthine (10^{-5}M) a P_1-purinoceptor antagonist (Burnstock 1978) did

Fig. 4.11. A: Trace showing the dual effect of ATP on joint blood vessels after elevation vascular tone by perfusion with 5HT (10^{-6}M). CNT represents the perfusion pressure prior to raising blood vessel tone. Small dots represent one minute intervals.

B: Vasodilator effect of adenosine (●) and ATP (■)on blood vessels with raised tone. During perfusion with the P_1-purinoceptor antagonist methylxanthine (10^{-5}M), the response to adenosine (O) was abolished (n = 7) but the response to ATP (□) was unaffected (n = 6).


A
20mmHg
Raised pressure
CNT
Baseline
Lock's ATP 10^{-2}M
B
100
50
0
*** *** *** NS NS NS
Perfusion pressure decrease (% of control)
-7 -6 -5 -4 -3 -2
Log [purine] (mol/l)
Adenosine
Adenosine + methylxanthine 10^{-5}M
ATP
ATP + methylxanthine 10^{-5}M

not affect the vasodilator action of ATP but abolished the response to injected adenosine (Figure 4.11A). As the dilator action of adenosine was unaffected by removal of the endothelial layer (Table 7), it is likely that the effects of adenosine were mediated via P_1-purinoceptors on the smooth muscle cells of the knee joint blood vessels. The vasoconstrictor effect of ATP was mediated via P_2-purinoceptors as perfusion of articular blood vessels with α , β methylene ATP, a potent P_2-purinoceptor desensitising agent, abolished the vasoconstrictor effect of ATP (Figure 4.12A). This vasoconstriction was unaffected by perfusion with methylxanthine but was slightly increased after removal of the endothelial layer (Figure 4.13).

The vasodilator action of ATP was mediated via the endothelium as pretreatment with deoxycholic acid (DCA) virtually abolished this response (Figure 4.12B). As the dilator response was not mediated via P_1-purinoceptors (Figure 4.11B), ATP may be acting via P_2-purinoceptors on the endothelial layer.

(iii). The effect of Acetylcholine (ACh)

Acetylcholine was found to have inconsistent effects on blood vessel calibre prior to elevation of vascular tone. In two preparations constriction was obtained, in one relaxation occurred and in a further three no effect was observed. However, when tone was elevated by perfusing with vasopressin (10^{-8}M), Ach then produced pronounced

Fig. 4.12. A: Constrictor response to injected ATP before (■) and during perfusion(□) with the P_2-purinoceptor desensitiser α,β methylene ATP (10^{-6}M). Responses in blood vessels prior to elevation of vascular tone (n = 7).

B: Dilator response to injected ATP before (■) and after removal of the endothelial layer (□) by DCA pretreatment. Vascular tone was elevated by perfusion with 5HT (10^{-5}M). n = 6-11.

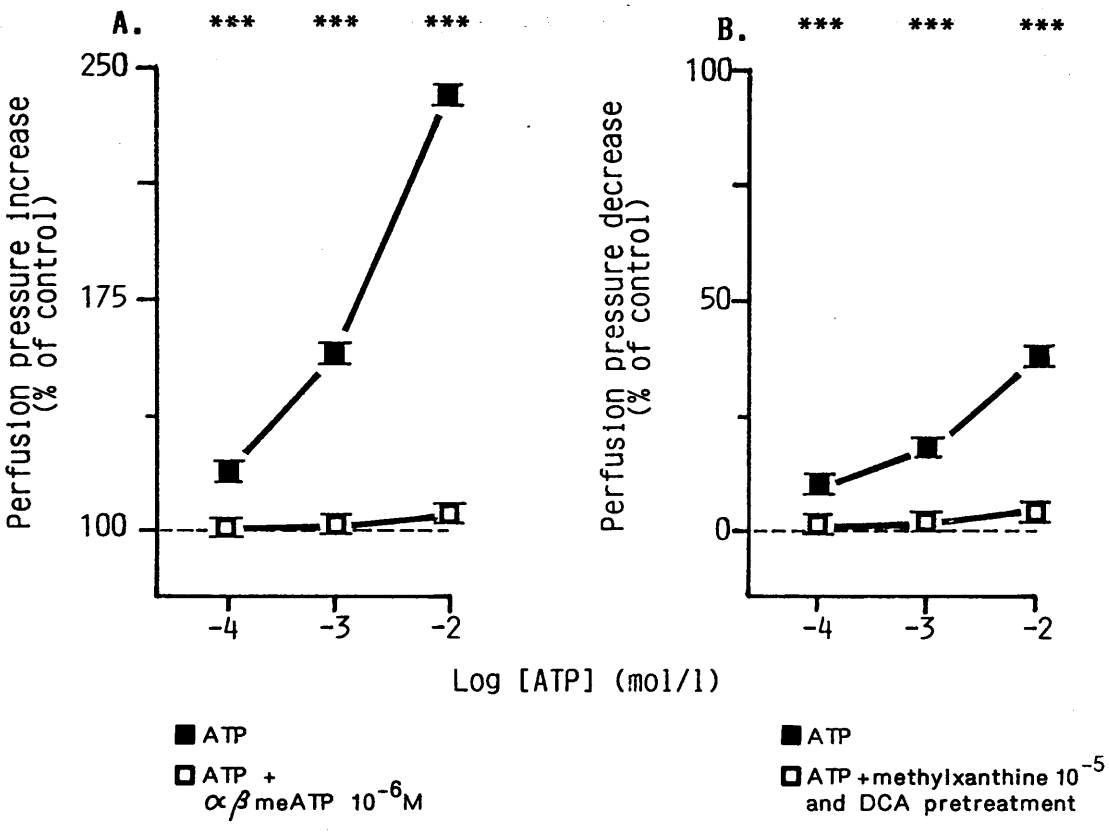
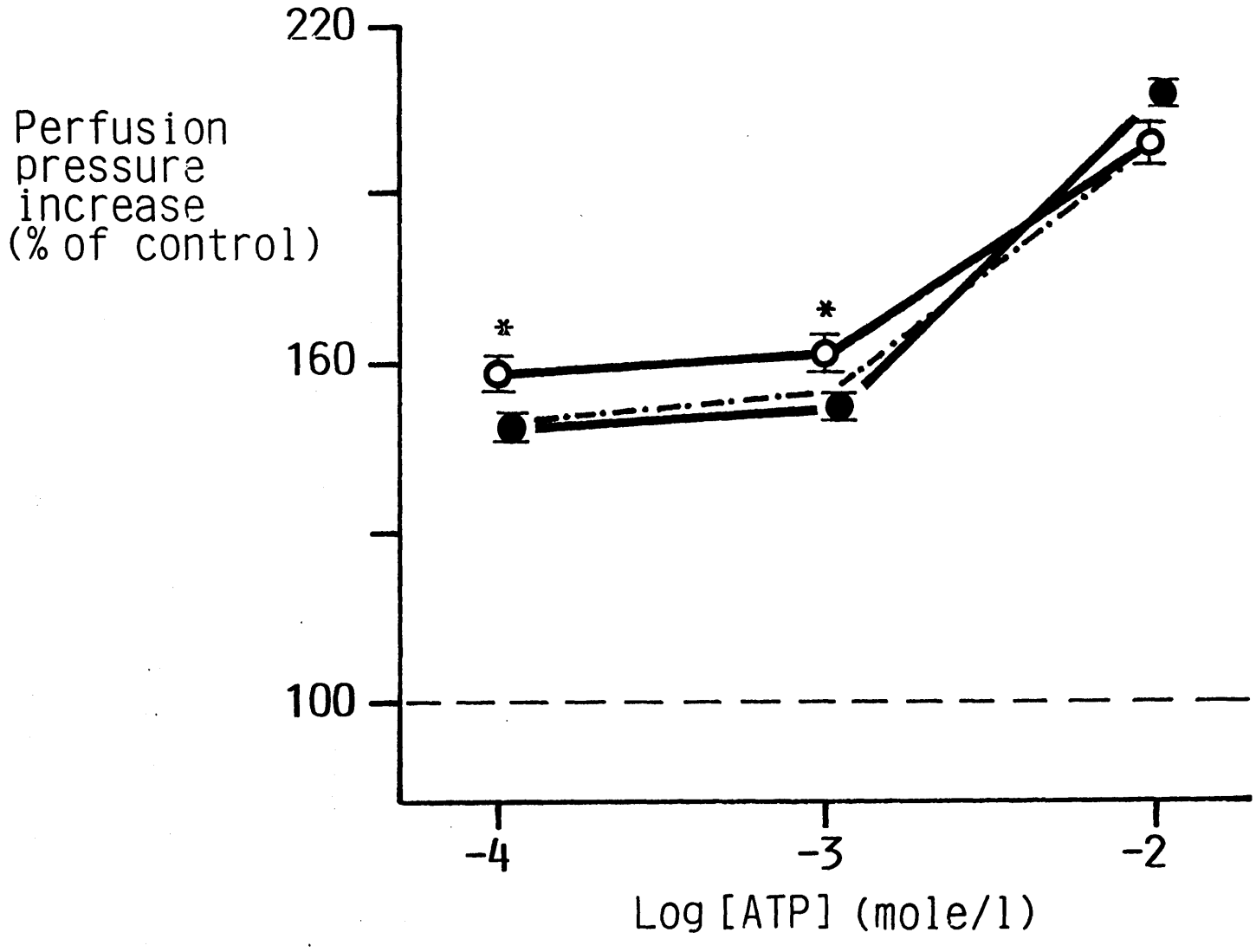

A.
***
***
***
250
175
100
Perfusion pressure increase (% of control)
-4
-3
-2
B.
***
***
***
100
50
0
Perfusion pressure decrease (% of control)
-4
-3
-2
Log [ATP] (mol/l)
ATP
ATP + αβmeATP 10-6M
ATP
ATP + methylxanthine 10-5 and DCA pretreatment

Fig. 4.13. The constrictor response to injected ATP before (●) and after removal of the endothelial layer by DCA pretreatment (O). The dot-dash line shows the response to ATP during perfusion with methylxanthine (10^{-5}M). In both cases vascular tone was elevated by perfusion with 5HT (10^{-5}M). n = 6 - 7.


220
160
100
Perfusion
pressure
increase
(% of control)
*
*
-4
-3
-2
Log [ATP] (mole/l)

vasodilation (Figure 4.14). This was mediated via muscarinic receptors as it was blocked by atropine (Figure 4.14).

The vasodilator action of Ach was dose-dependent (10^{-6}M to 10^{-4}M) and the receptors mediating this effect were located on the vascular endothelial layer as its removal virtually abolished the actions of Ach (Figure 4.14B).

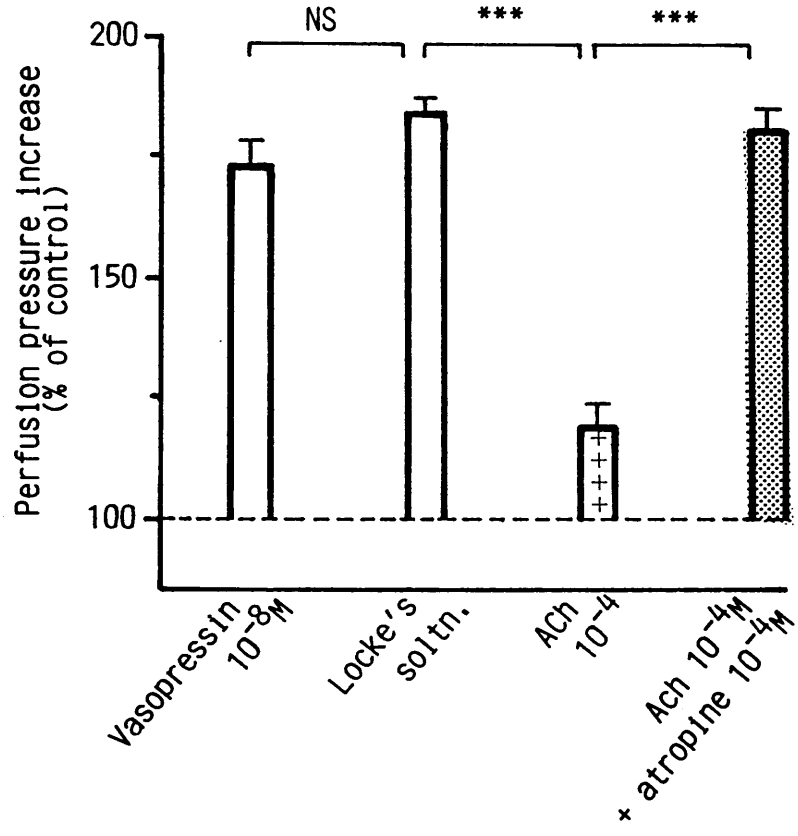
c. The effect of Histamine in-vivo

Although it is well known that histamine acts on blood vessels to induce vasodilation and increase the permeability of capillary beds (Haddy 1960, Powell and Brody 1976), there are reports indicating that histamine can raise the tone of vascular smooth muscle cells in some areas (Furchgott 1981, Kennedy and Burnstock 1985). In present experiments intra-arterial injection of histamine (10^{-10}M to 10^{-5}M) has been tried. It induced vasoconstriction in articular blood vessels (Figure 4.15) which was dose dependent. This response was reduced with injection of diphenhydramine prior to histamine injection but not with cimetidine, suggesting the effect was mediated mostly via H_1 receptors (Figure 4.16).

This effect was independent of the systemic blood pressure changes, when the blood flow to the experimental limb was isolated by perfusion technique, although there is the possibility that histamine might dilate other blood vessels, thus shunting blood flow from joint blood vessels

Fig. 4.14. A: (upper panel) Dilator effect of injected acetylcholine (n = 9) and the abolition of this effect by atropine (n = 9) in blood vessels whose tone had been raised by vasopressin (10^{-8}M).

B: (lower panel) The dilator effect of acetylcholine (n = 9 - 12) before (■) and after removal of the endothelial layer (□) by DCA pretreatment (n = 6 - 8). Vascular tone was raised by vasopressin (10^{-8}M).


NS
***
***
200
150
100
Perfusion pressure increase
(% of control)
++++
Vasopressin
10⁻⁸M
Locke's
soltn.
ACh
10⁻⁴
Ach 10⁻⁴M
+ atropine 10⁻⁴M

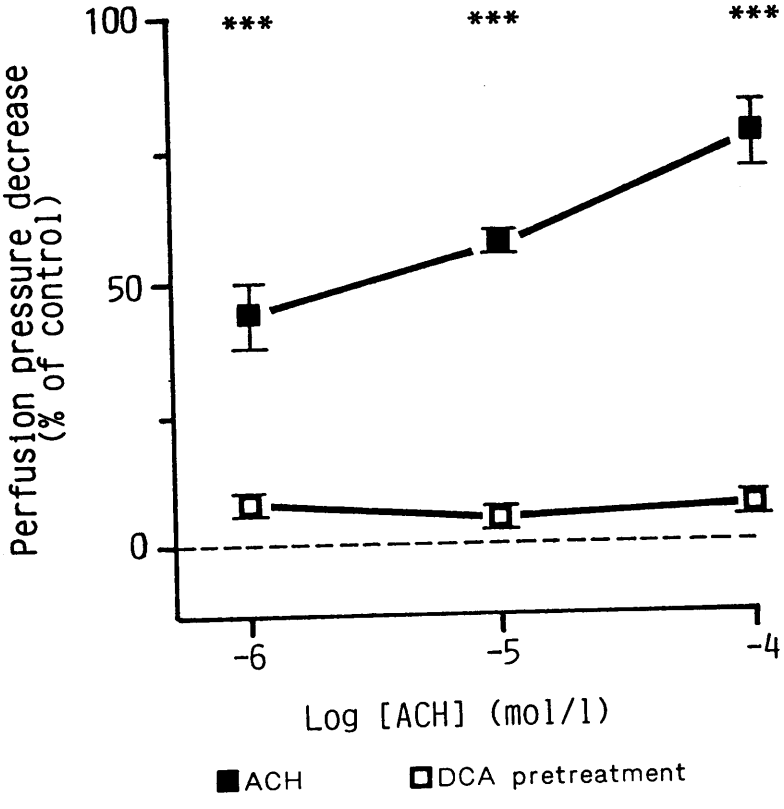
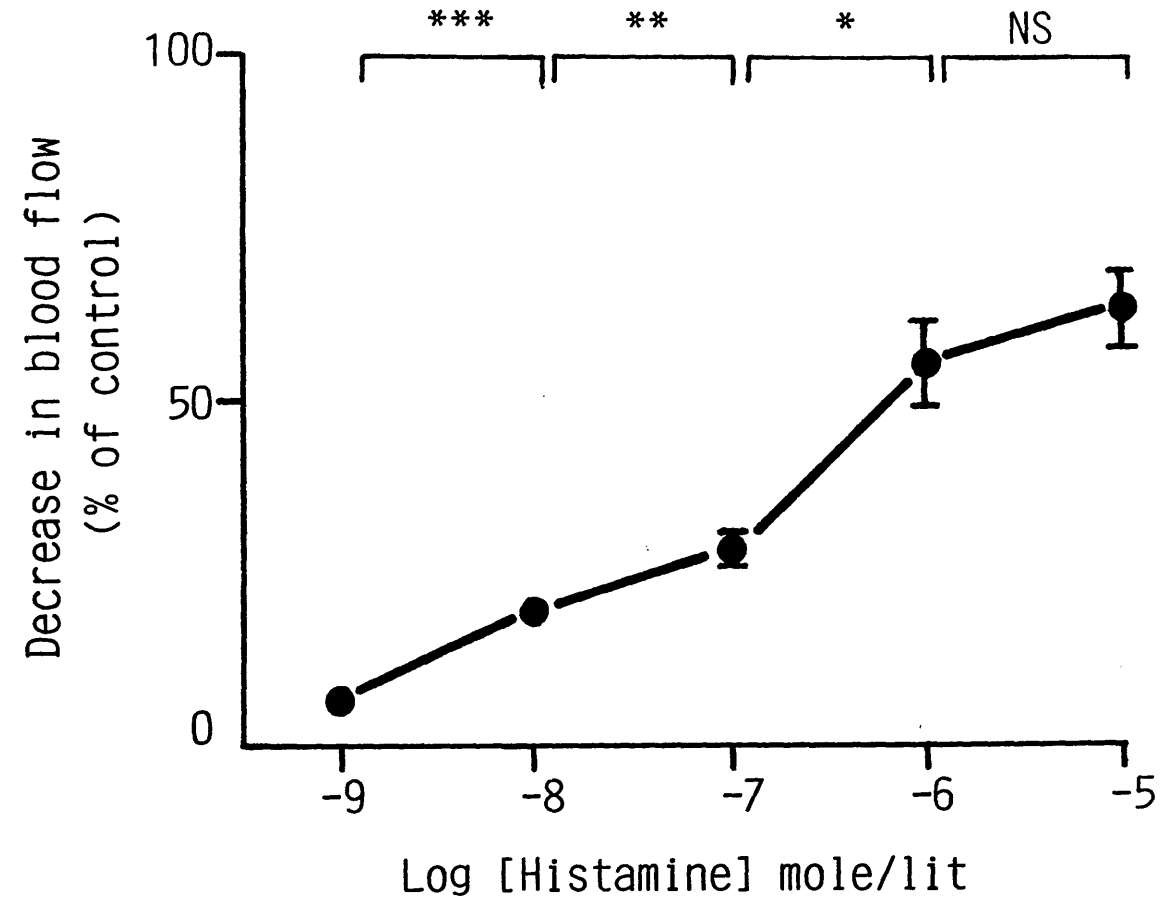
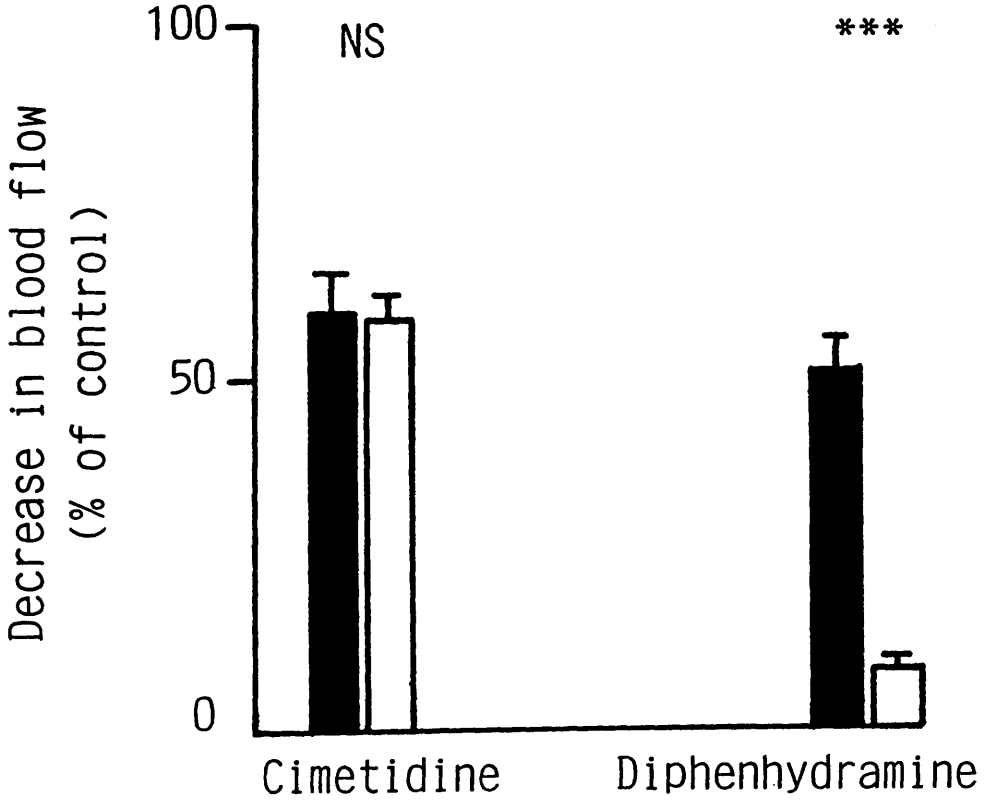

100
50
0
***
***
***
Perfusion pressure decrease
(% of control)
-6
-5
-4
Log [ACH] (mol/l)
ACH
DCA pretreatment

Fig. 4.15. The dose-dependent effect of histamine on aricular blood vessels in rabbit. Histamine induce vasoconstriction in these vessels. n=5-6, NS; Non significant, * P<0.05, ** P<0.01, *** P<0.001.

Fig. 4.16. The effect of diphenhydramine (10^{-4}M) and cimetidine (10^{-4}M) on the response of articular blood vessels to histamine injection. Filled histograms show the response of histamine (10^{-4}M), which the open histogram show the response to the same injection after pre-treatment with a H_2 antagonist (Cimetidine) and a H_1 antagonist (Diphenhydramine). n= 5 NS; Non significant, *** P<0.001.


***
**
*
NS
100
50
0
Decrease in blood flow
(% of control)
-9
-8
-7
-6
-5
Log [Histamine] mole/lit


100
50
0
Decrease in blood flow
(% of control)
NS
***
Cimetidine
Diphenhydramine

to these other areas. But it should be remembered that histamine was used to raise the vascular tone in the in-vitro preparations where all other blood vessels were ligated except knee joint vessels. These results suggest that even if histamine increases the capillary permeability in the joint capsule it can have independent effects on vascular smooth muscle cells.

DISCUSSION

The purpose of this section was twofold. Firstly, to establish whether purinergic receptors are present in rabbit knee joint blood vessels and determine the effects they mediate. Secondly, to investigate whether any actions of purinergic receptors are mediated via the endothelial layer of articular blood vessels.

Burnstock (1978) proposed that there were two subtypes of purinoceptors: P_1-purinoceptors whose interaction with adenosine can be antagonised by methylxanthines and P_2-purinoceptors which are more sensitive to ATP and can be antagonised by α , ß methylene ATP (Burnstock, 1982; Kasakov & Burnstock, 1983). Research on various tissues in different species indicates that purine nucleotides act via P_1-purinoceptors to induce relaxation of vascular smooth muscle cells (Burnstock 1982, 1986) but that actions via P_2-purinoceptors produce contraction in some tissues and relaxation in others (Burnstock & Brown, 1981; Burnstock & Kennedy, 1985).The relaxation of smooth muscle cells can either be direct via receptors on smooth muscle cells or indirectly via receptors on the endothelial layer (Furchgott, 1981).

The results of the present experiments demonstrate the dual effect of ATP on articular blood vessels in the rabbit. ATP can produce vasoconstriction via

P_2-purinoceptors on vascular smooth muscle. The desensitisation action of α ,β methylene ATP which has been described previously (Kasakov & Burnstock 1983; Hedlund, Fandriks, Delbro & Fasth 1983; Sneddon & Burnstock 1984; Kennedy & Burnstock 1985; Kennedy Delbro & Burnstock 1985) was confirmed here. Removal of the endothelium resulted in a slight increase in the vasoconstrictor action of ATP suggesting that the responses prior to DCA treatment were reduced by continuous release of endothelium derived relaxation factor (EDRF).

In contrast to ATP, adenosine did not produce any constrictor effect. However, after increasing the tone of joint blood vessels it caused concentration-dependent relaxation. This response was mediated via P_1-purinoceptors on vascular smooth muscle as it was antagonised by methylxanthine, but unaffected after removal of the endothelium. The weak effect of adenosine prior to elevation of vascular tone was probably due to this agent acting on blood vessels which were already vasodilated in the absence of tonic sympathetic constrictor discharge.

Acetylcholine was also found to exert a dilator effect which was consistently observed after increasing the vascular tone. This was, however, virtually abolished by removal of the endothelium, which is similar to the results obtained by Furchgott & Zawadski (1980) on rabbit thoracic aorta.

Contrary to an earlier report based on rabbit ear

artery by Kennedy & Burnstock (1985), ATP appears to produce vasodilation via P_2-purinoceptors, as methylxanthine fails to block this effect whereas removal of the endothelium does. This finding is in agreement with another study on rat femoral artery carried out by Kennedy et al (1985). Whether the dilation is due to a direct action of ATP, or whether it is mediated by the breakdown products of ATP such as AMP and adenosine (Burnstock 1978), remains to be answered. It is possible that these act via P_2-purinoceptors on endothelial cells, although previous reports have suggested that P_2-purinoceptors appear to play little or no role in ATP-elicited vasodilation (Su, 1981; Kennedy & Burnstock, 1985).

The physiological significance of the different distribution of P_1 and P_2 purinoceptors in different vascular beds remains unclear (Kennedy & Burnstock 1985). However, it is likely that these purinoceptors and the endothelium may prove to have an important role in the regulation of the blood flow through articular blood vessels.

****

****

****

****

***

*

SECTION II

NERVOUS CONTROL OF ARTICULAR BLOOD VESSELS IN THE RABBIT

SUMMARY

1.An *in vitro* preparation of the rabbit knee joint, perfused with oxygenated Locke's solution, was used to study the response of articular blood vessels to electrical stimulation of the joint capsule.

2.Articular blood flow changes were also recorded in response to saphenous nerve stimulation *in-vivo*.

3.Using trains of stimulus pulses of different durations, frequency response curves were obtained. Electrical stimulation always produced vasoconstriction of joint blood vessels, which increased as a function of both frequency and pulse width.

4. Stimulation of the saphenous nerve resulted in an attenuation of articular blood flow during stimulation, followed by a prolonged vasodilation on cessation of stimulation in many preparations.

5.*In-vitro*, the vasoconstrictor response was neurally mediated as it was markedly inhibited after addition to both bath and perfusate of tetrotodoxin, and in animals pretreated with reserpine.

6.The response to electrical stimulation (in-vitro)

and saphenous nerve stimulation (in-vivo)was substantially reduced by the α -adrenergic antagonists phenoxybenzamine and phentolamine (10^{-5}M), the α_1 -blocker prazosin (10^{-6}M), and by guanethidine (10^{-5}M) which inhibits the release of noradrenaline, ATP, and neuropeptide Y from sympathetic nerve endings.

7.<u>In-vitro</u>, the attenuation of the vasoconstrictor response to field stimulation by prazosin (10^{-6}M) was little altered by addition of the α_2 adrenoceptor blocker rauwolscine (10^{-6}M) to the perfusate.

8. α , β methylene ATP (10^{-6}M), a P_2-purinoceptor desensitiser, had no effect on the vasoconstrictor response to electrical stimulation.

9.The dilator response which followed the initial vasoconstriction was blocked by the substance P antagonist (D-Pro^4,D-$Trp^{7,9,10}$)-SP(4-11).

10.These results suggest that the vasoconstrictor response to electrical stimulation of sympathetic nerve fibres in the rabbit knee joint capsule is mediated via noradrenaline acting upon α_1 -adrenoceptors, and substance P may be the mediator of the dilator response to saphenous nerve stimulation.

INTRODUCTION

Although the somatic innervation of joints has received much attention (for review see Gardner 1950), little is known about the autonomic control of articular blood vessels. Cobbold and Lewis (1956) suggested that these are innervated by sympathetic efferent fibres as they observed that electrical stimulation of the sympathetic chain resulted in a reduction of blood flow to the dog knee joint. In a recent study on the cat knee, electrical stimulation of the posterior articular nerve to this joint, which is known to contain sympathetic efferent fibres (Langford & Schmidt 1982), produced an initial vasoconstriction of the blood vessels followed by a prolonged vasodilation (Ferrell and Cant 1987). However, the nature of the neurotransmitters and receptors which mediate the vasoconstrictor or vasodilator responses was not investigated in either of these studies.

Following the work of Ahlquist (1948), catecholamine receptors mediating constriction of arteries have been designated α -adrenoceptor. It was widely assumed that these must be the only post-synaptic receptors despite the observation that many α_1 -receptor antagonist fail to block nerve-induced vasoconstriction (Neild and Zelcer 1982). Hirst and Neild (1980) suggested that the electrical and mechanical responses of some smooth muscles

were resistant to α_1 -adrenoceptor antagonists because neuronally released noradrenaline was acting not only on α_1 -adrenoceptors but also on a new class of adrenoceptors which they designed γ -receptors located near the nerve-muscle junction. However, other studies indicated that the α -blocker-resistant portion of the contractile response to sympathetic nerve stimulation was mediated by ATP, acting either as a co-transmitter with noradrenaline (Nakanishi and Takeda 1972; Burnstock 1976; Langer and Pinto 1976; Westfall, Stitzel & Rowe 1978; Sneddon and Burnstock 1984), as a neuromodulator (Su 1977, Wakade and Wakade 1978; De May, Burnstock & Vanhoutte 1979; Moylan & Westfall 1979), or as a neurotransmitter in its own right (Burnstock, Campbell, Statchell & Smythe 1970).

The suggestion that some nerve cells store and release more than one transmitter was made by Burnstock (1976), largely on the basis of comparative studies of the evolution of the autonomic nervous system (Burnstock 1969). Su (1975,1978) used tritium-labelled adenosine and noradrenaline to show that ATP is released together with noradrenaline from sympathetic nerves supplying the rabbit aorta and portal vein. Co-existence of noradrenaline and ATP has also been demonstrated in the rabbit ear artery (Head, Stitzel, Delaland & Johnson 1977), and in the dog basilar artery (Muramatsu, Fuginara, Muira, & Sakakibara, 1981). In the rat tail artery, electrical responses to sympathetic nerve stimulation consist of two components namely a fast depolarization blocked by α,β methylene

ATP, suggesting mediation by P_2-purinoceptors and a slow maintained depolarization which was blocked by phentolamine, suggesting that this is mediated by α-adrenoceptors (Burnstock, Griffith & Sneddon 1984). Since then considerable evidence has accumulated in support of the multiple transmitter concept including electron microscopic evidence of more than one type vesicle in sympathetic nerve endings (Burnstock 1986a).

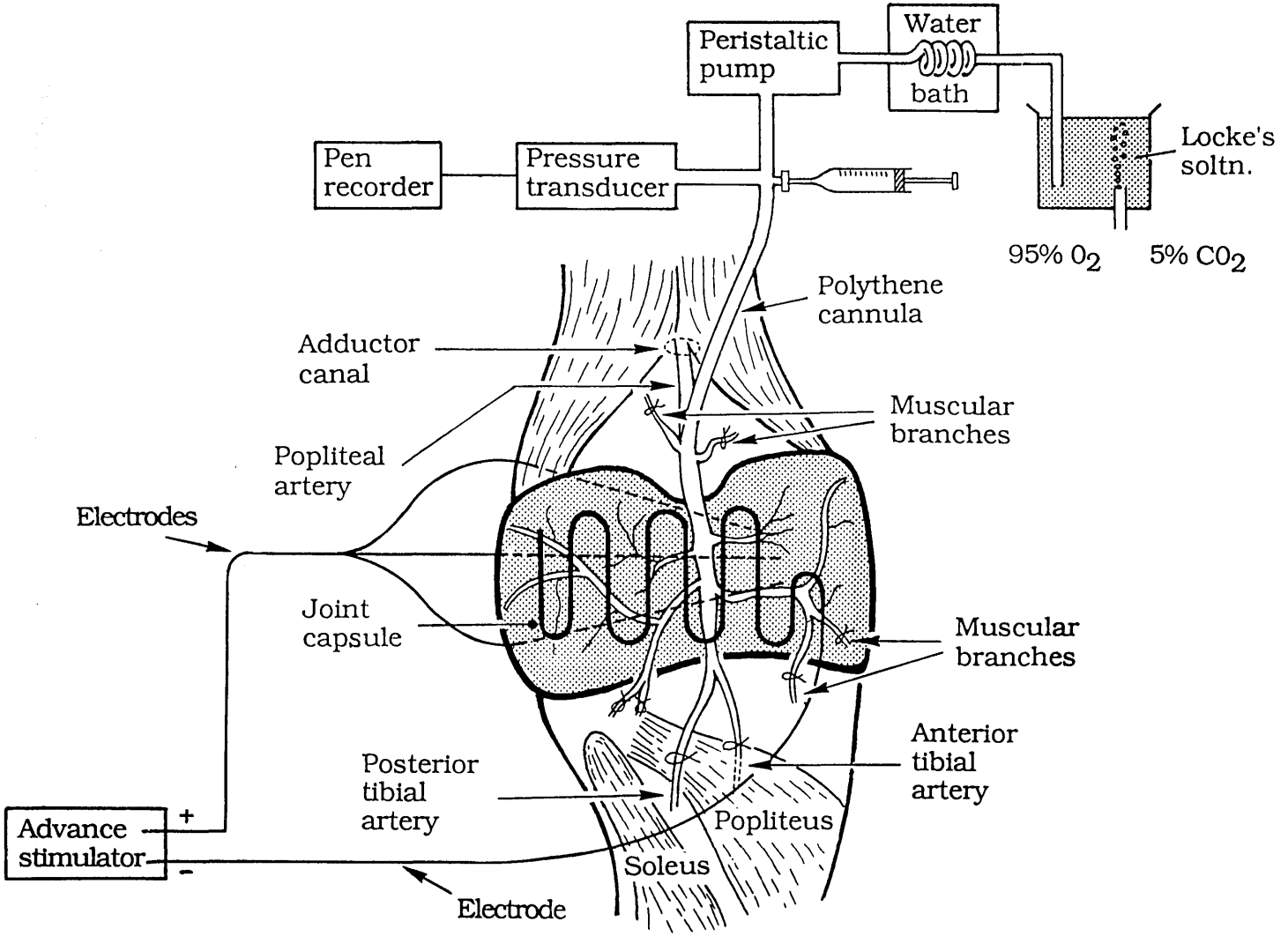
Thus, in many tissues noradrenaline and ATP or other substances may be co-transmitters but whether this also applies to articular blood vessels is not known. In the present study, this possibility was investigated by assessing the effects of α-adrenoceptor blockers such as phenoxybenzamine and phentolamine (α_1, α_2 antagonists, Weiner 1985), prazosin (selective α_1 antagonist), rauwolscine (selective α_2 antagonist), UK-14304 (selective α_2 agonist), drugs such as guanethidine which inhibit release of noradrenaline, ATP, and neuropeptide Y from sympathetic nerve endings (Lundberg, Anggard, Theodorsson-Noheim & Pernow, 1984), α,β methylene ATP, a P_2-purinoceptor desensitiser, and a substance P antagonist on the responses of blood vessels in the rabbit knee to electrical stimulation of the joint capsule.

EXPERIMENTAL PROTOCOL

a: Field stimulation in-vitro

Experiments were performed on albino New Zealand rabbits of either sex weighing between 2-3.3 kg, which were killed by stunning and exsanguination. Immediately thereafter, the knee were prepared for in-vitro recording explained previously(Chapter two). The isolated knee joint preparation was then transferred to a thermostatically controlled bath (37 $\pm$ 1°C) containing oxygenated Locke's solution. Three silver chloride wire electrodes were inserted via 21G hypodermic needles into the synovial cavity (Figure 4.17) which was injected with 1ml of Locke's solution. The central ends of these wires were connected together to the anodal output of the stimulator (Harvard Advanced Stimulator). The cathodal output was connected to a silver chloride wire electrode shaped to form a zig-zag pattern, which overlay and made contact with the dorsal aspect of the knee joint capsule. At the start of the experiment the pump was set to provide a perfusion rate of 0.5-0.9ml/min which resulted in a perfusion pressure of about 40-50 mmHg as measured by a pressure transducer connected "downstream" from the pump. After a 30 min equilibration period a steady resting perfusion pressure was achieved. Thereafter, any fall in perfusion pressure as a result of field stimulation

Fig. 4.17. Digrammatic representation of the relations between the location of the arterial supply of the dorsal aspect of the rabbit knee joint capsule and the silver chloride electrodes.


Peristaltic pump
Water bath
Pen recorder
Pressure transducer
Locke's soltn.
95% O_2
5% CO_2
Polythene cannula
Adductor canal
Muscular branches
Popliteal artery
Electrodes
Joint capsule
Muscular branches
Anterior tibial artery
Posterior tibial artery
Popliteus
Advance stimulator
+
-
Soleus
Electrode

provided an indirect measure of articular vasoconstriction.

Peak response was compared to control (pre-injection) value and expressed as percentage change from control (or baseline). In most experiments the stimulus pulse width and voltage were varied until the optimal response was obtained and thereafter maintained constant. In most instances the stimulus parameters were: frequency 10 pulses/sec, voltage 40V and pulse width 1msec. In all instances the stimulus pulse train lasted 30 sec. Once suitable stimulus parameters were established, different adrenoceptor and purinoceptor blockers, dissolved in Locke's solution, were perfused continuously for periods of up to two hours, and at different time intervals the electrical stimulus was re-applied. Thereafter, the perfusate was switched back to Locke's solution and the whole organ bath solution was changed several times.

In order to deplete sympathetic nerve ending of noradrenaline, two rabbits were injected with 0.2mg/kg/day reserpine into the ear vein on three successive days and on the fourth day they received a final dose of 1mg/kg reserpine (ip), then 5 hours after this last injection the animal was killed by a blow to the skull and exanguinated, and the rest knee joint prepared as describe earlier (Methods).

b: Saphenous nerve stimulation in-vivo

White New Zealand rabbit weighing 2.1-3.4 kg deeply anaesthetised with a mixture of intraperitoneal Diazepam (0.5mg/kg) and intramuscular hypnorm (0.1mg/kg), were used Anaesthesia was maintained by giving 1-2% halothane in O_2, N_2O which was delivered via a tracheal cannula. The carotid artery was cannulated for measurement of blood pressure. A cannula was inserted in a retrograde direction in saphenous artery below the knee joint and advanced until its tip was just distal the branches supplying the knee joint.

The saphenous nerve was dissected free from the adjacent blood vessels. Then the thigh was held at a fixed position and a thigh paraffin pool was prepared. After the saphenous nerve was sectioned proximally, it was located on bipolar silver chloride electrodes, and this was connected to an Harvard Advanced stimulator.

To observe relative changes in blood flow either a near infra-red (780nm) laser Doppler flowmeter (MBF2 Moor Instruments) or a modified laser Doppler flowmeter (MBF3 Moor Instruments) used. The MBF3 instrument used a special triple fibre-optic probe which permitted use of three wavelenghts of laser radiation. The probe connected to the MBF2 was placed on the surface of the capsule anteriorly, and the MBF3 probe was inserted from the antero-lateral aspect of knee joint into the joint cavity to make contact

with the internal surface of the antero-medial infra-patellar region.

In most cases the stimulus parameters were: Frequency 10 pulses/sec, Voltage 10 Volts, and Pulse width 1 msec. The stimulus pulse train lasted 30 seconds. Once the suitable constrictor responses were obtained 0.2ml of different adrenoceptor and purinoceptor blockers were injected.

RESULTS

a: Responses of articular blood vessels to field stimulation

Electrical stimuli applied to the joint capsule invariably resulted in an increase in perfusion pressure, indicating vasoconstriction (Figure 4.18 left panel). Increase in stimulus voltage enhanced the vasoconstrictor response but did not change its form.

To assess whether field stimulation mediates its vasoconstrictor effect solely via the sympathetic efferent nerve fibres surrounding joint blood vessels, or whether the smooth muscle cells in these vessels are also affected, tetrotodoxin (TTX) was used. This has been used in other experiments to block neuronal conduction (Stevens & Moulds 1985; Burnstock 1986b). TTX (10^{-7}M) was perfused for 20 min and was also added to the organ bath (10^{-8}M). As shown in figure 4.18 middle panel and 4.19, TTX markedly inhibited the response to field stimulation and this could be reversed by perfusion with fresh Locke's solution and repeated changes of the organ bath (figure 4.18 right panel). However, lack of complete blockade could be due to the dose of TTX used which is unable to block all unmyelinated nerve fibres(Kirchhoff et al 1989).

In other reserpinised animals, as illustrated in figure 4.20, although intra-arterial injection of

Fig. 4.18. The effect of field stimulation of the dorsal aspect of the joint capsule (left panel). 20min after perfusion with TTX (10^{-7}M) and with TTX in the bath (10^{-8}M) the response to stimulation is markedly reduced (middle panel). 20min after terminating TTX treatment, response to stimulation returns(right panel). The top trace indicates stimulus pulse train. Stimulus parameters throughout: 50V, 10 pulses/sec, 1msec.

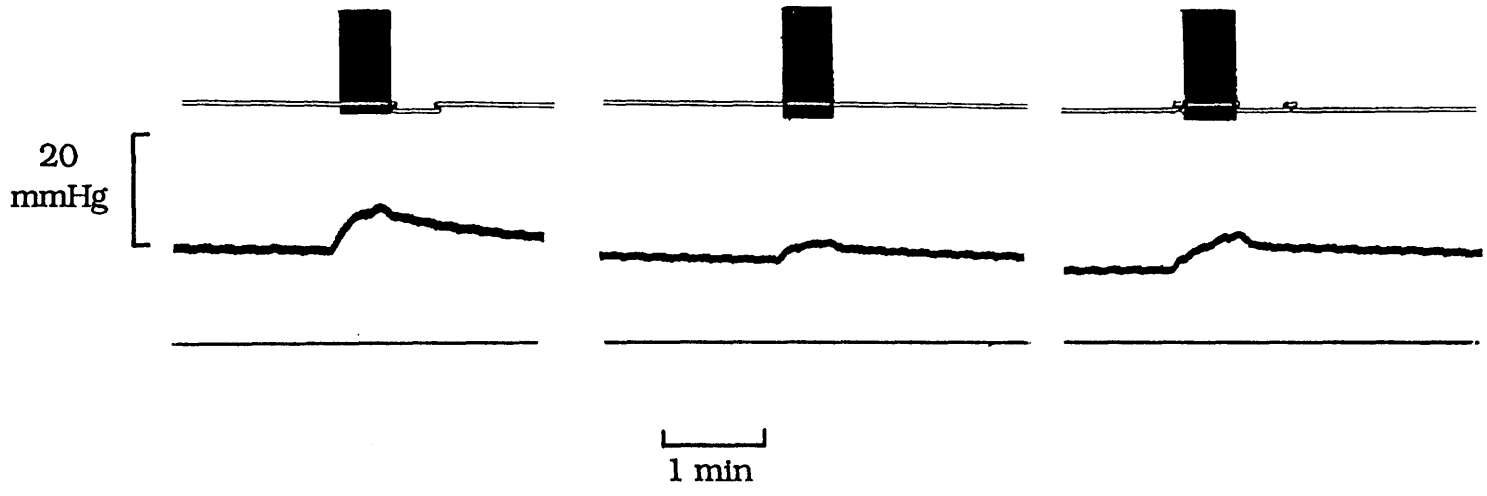

20
mmHg
1 min
Before
TTX
perfusion
During
TTX
perfusion
30' after
TTX
perfusion

Fig. 4.19. The effect of field stimulation of dorsal aspect of the joint capsule with different pulse widths (0.1, 0.5, 1, 2msec.) before TTX perfusion (upper trace) and after perfusion with TTX (10^{-7}M) and with TTX in bath (10^{-8}M). The responses to field stimulation is markedly reduced (lower trace).

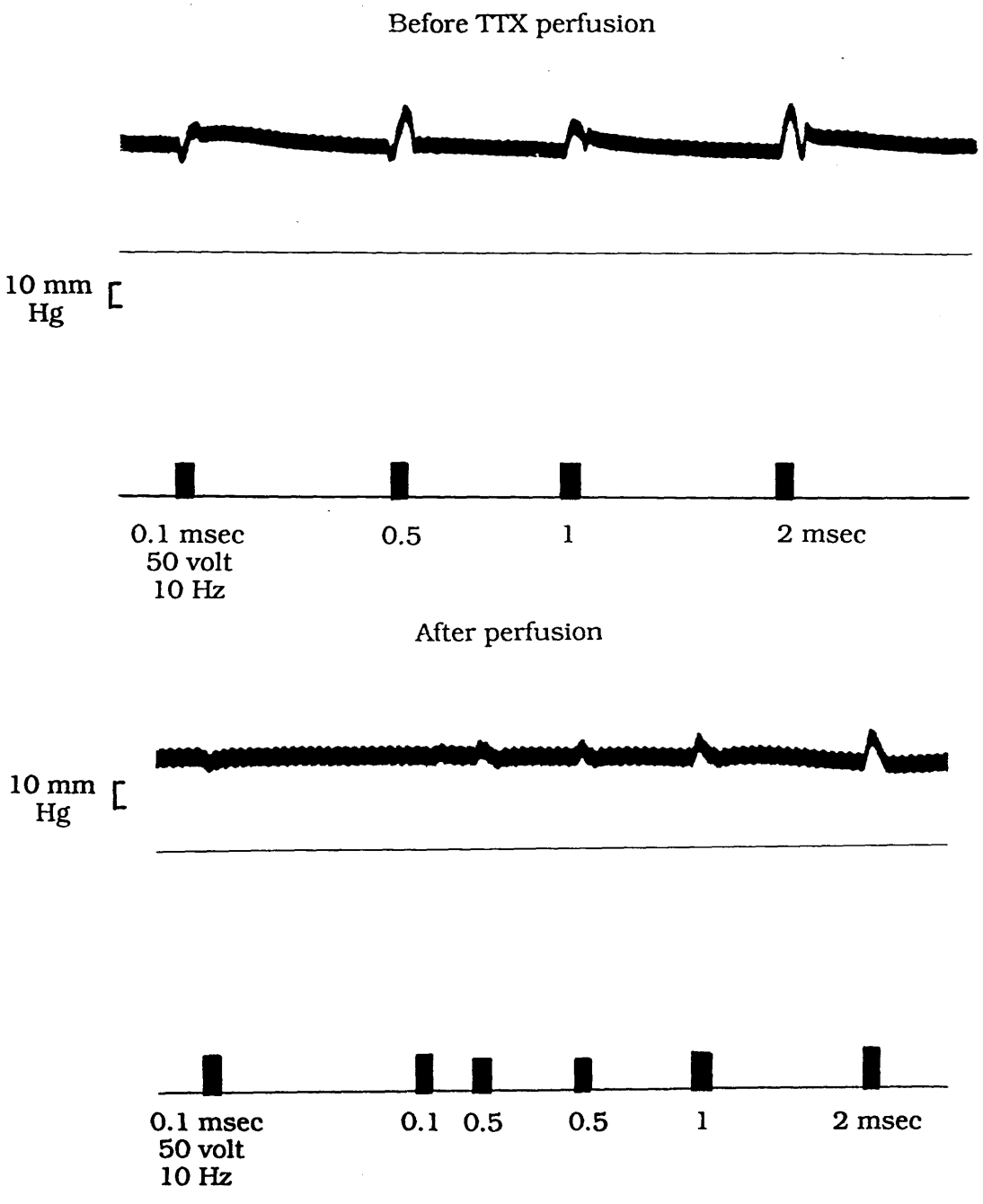
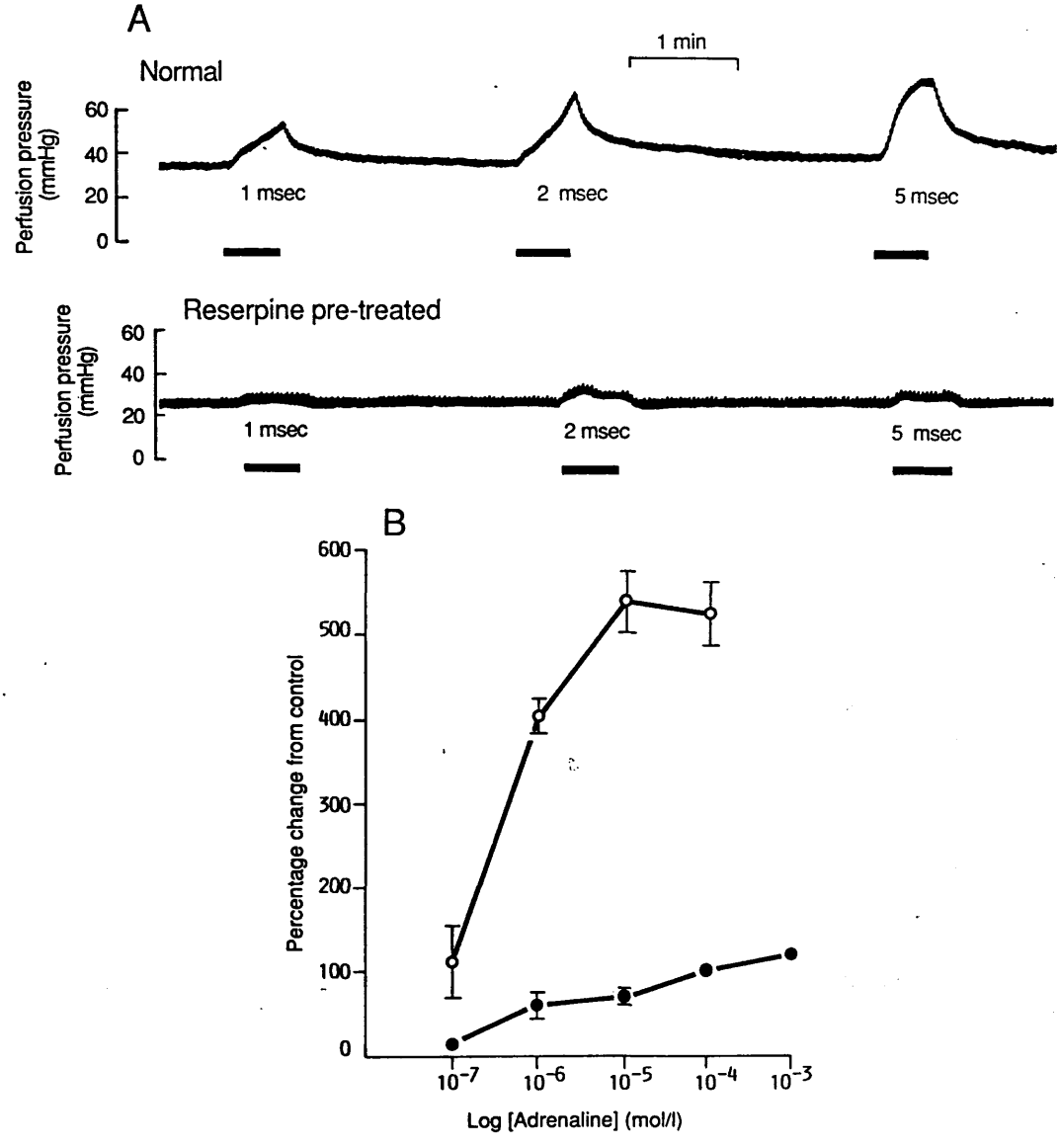

Before TTX perfusion
10 mm Hg
0.1 msec
50 volt
10 Hz
0.5
1
2 msec
After perfusion
10 mm Hg
0.1 msec
50 volt
10 Hz
0.1
0.5
0.5
1
2 msec

Fig. 4.20. A: The effect of field stimulation of the posterior capsule in normal (upper trace) and reserpinized animals(lower trace) with different pulse widths (1, 2, 5, msec). Other stimulus parameters were kept constant, (40V, 20 HZ, and 30sec duration).

B: Changes in perfusion pressure (mean ± SEM) with increasing doses of adrenaline in normal (●) and reserpinized rabbit (O). n=4-6.

The concentrations can be converted to the mass of drug injected by multiplication of that concentration by 2×10^{-4} in this and subsequent figures.


A
Normal
1 min
Perfusion pressure (mmHg)
60
40
20
0
1 msec
2 msec
5 msec
Reserpine pre-treated
Perfusion pressure (mmHg)
60
40
20
0
1 msec
2 msec
5 msec
B
Percentage change from control
600
500
400
300
200
100
0
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
Log [Adrenaline] (mol/l)

adrenaline showed that depletion of nerve ending enhances the sensitivity of adrenoceptors and the responses to field stimulation were exaggerated, but the responses to field stimulation with 1, 2, and 5 msec pulse width (36.3 ± 2.0, 52.7 ± 1.6, 108.5 ± 6.7 respectively) were still significantly reduced in the reserpinised animals(9 ± 2.4, 13.8 ± 2.2, 16.9 ± 2.6 respectively). This suggests that the stimuli applied are sufficient to affect the nerves supplying the joint but have little direct effect on vascular smooth muscle.

Responses to field stimulation were obtained both by increasing the number of pulses per second and also by increasing the pulse width (Figure 4.21). These show considerable enhancement of the constrictor responses which were all inhibited when the joints were perfused with phenoxybenzamine (10^{-5}M) for about one hour before stimulation.

b: Changes in articular blood flow due to stimulation of the saphenous nerve

After the dissection procedures and locating the laser probe in position, the saphenous nerve was sectioned proximally. As a result, in some animals the articular blood flow increased (Figure 4.22), presumely the result of loss of sympathetic tone to articular blood vessels.

By direct electrical stimulation of the saphenous nerve, a characteristic pattern of initial fall in blood

Fig. 4.21. Responses to field stimulation before (closed symbols) and during perfusion with phenoxybenzamine at 10^{-5}M (open symbols). Three pulse widths were used: 5msec (●), 2msec (▲) and 1msec (■). During perfusion with phenoxybenzamine the responses to electrical stimulation were virtually abolished at all three pulse widths and hence the symbols are superimposed. n = 6 - 10.

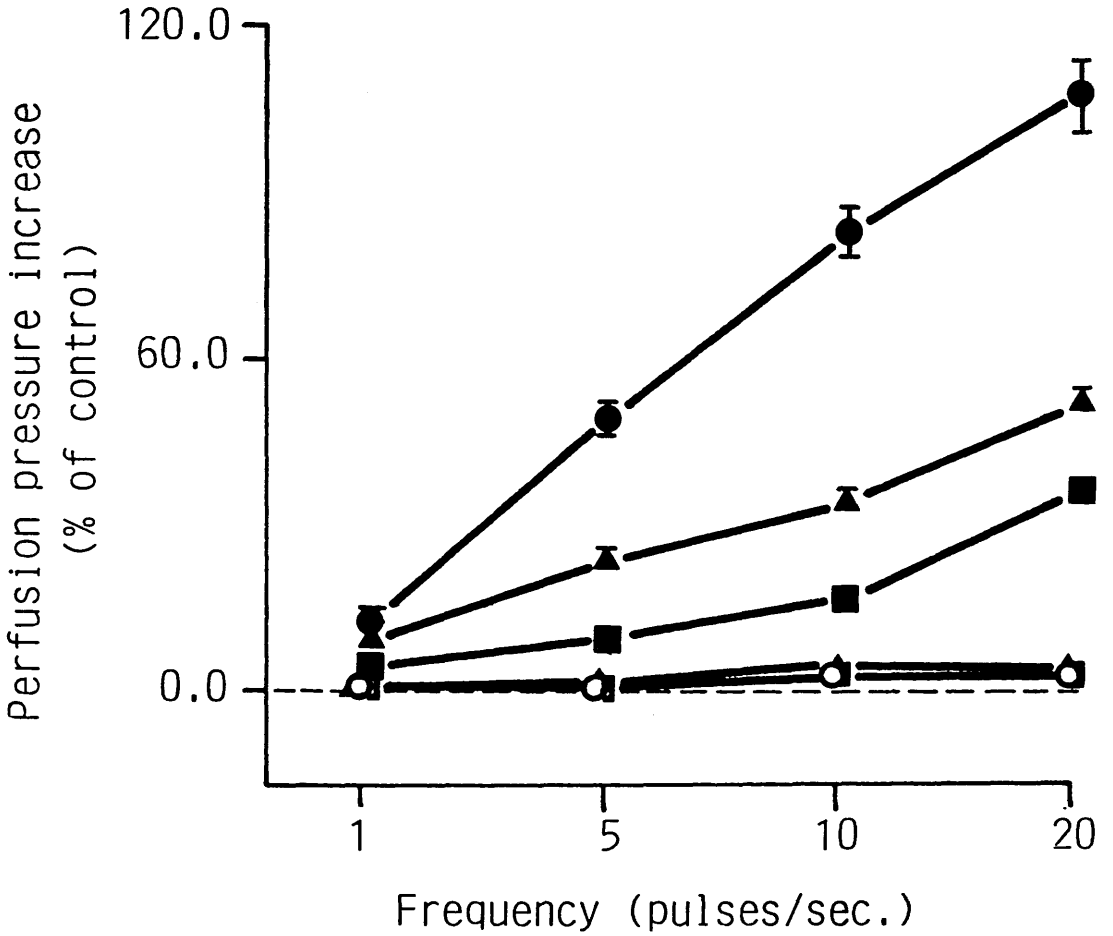
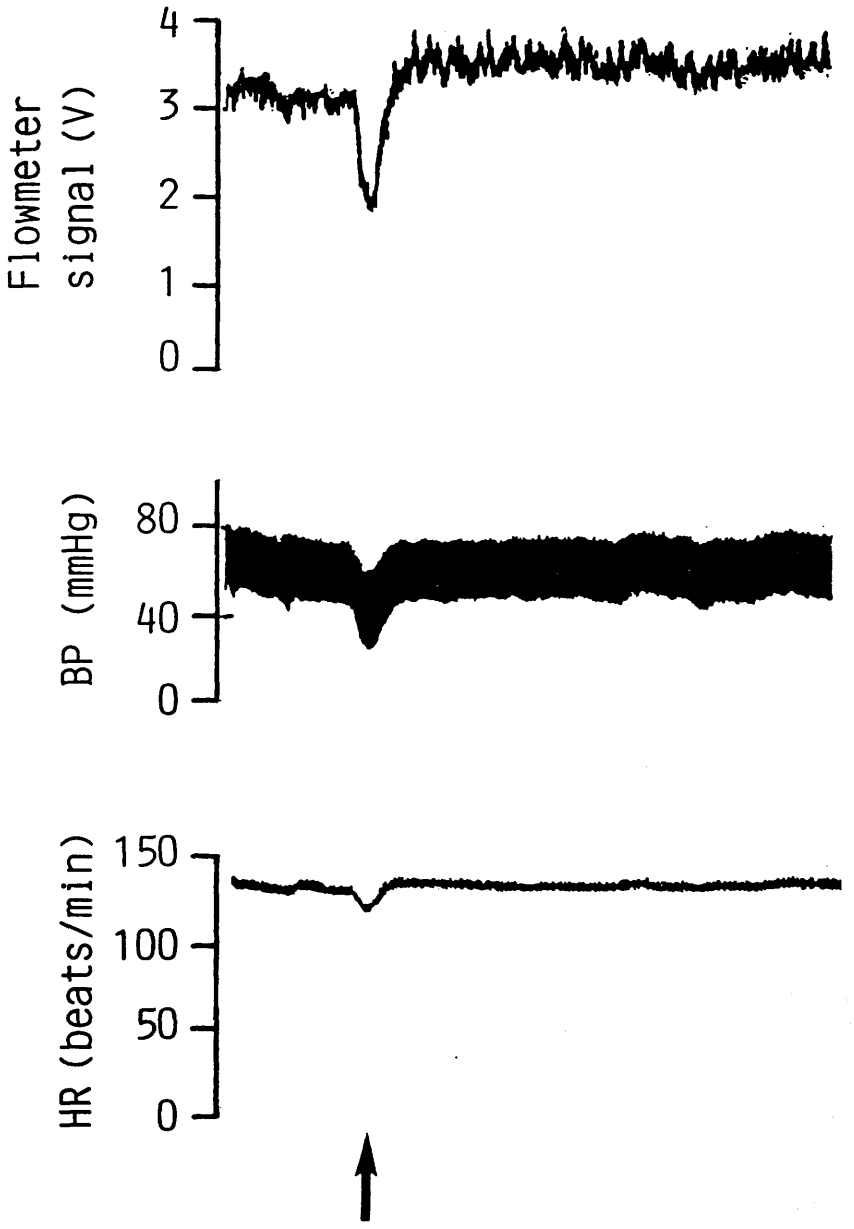

120.0
60.0
0.0
Perfusion pressure increase
(% of control)
1
5
10
20
Frequency (pulses/sec.)

Fig. 4.22. Trace shows the effect of transection ofthe rabbit saphenous nerve on the articular blood flow. Arrow shows the time that the nerve was sectioned. Rise in the flowmeter signal even when the blood pressure returned close to its original value indicates vasodilation which might be due to removal of sympathetic tone from these blood vessels.


Flowmeter signal (V)
4
3
2
1
0
BP (mmHg)
80
40
0
HR (beats/min)
150
100
50
0

flow of the joint during the period of stimulation in all animals followed by a prolonged rise in articular blood flow (12 out of 16 experiments) on cessation of stimulation (Figure 4.23). This dilator response followed after the initial constrictor response was significantly reduced by an intra-articular injection of 100ug substance P antagonist ($D\text{-}Pro^{4}, D\text{-}Trp^{7,9,10}$)-SP(4-11) before stimulating the saphenous nerve (Figure 4.24). In few animals it was found that no rise in articular blood flow occurred on saphenous nerve stimulation , although the initial fall in blood flow was present (Figure 4.25).

Increasing the frequency of saphenous nerve stimulation showed considerable enhancement of constrictor responses(Figure 4.25, 4.26). the responses were maximal at 30 pulses per second and decreased thereafter.

c: Nature of neurotransmitters mediating the constrictor response

In order to determine the nature of the neurotransmitters mediating the vasoconstrictor response, the effect of electrical stimulation was observed in the in-vitro preparation during perfusion with the α_1, α_2 adrenoceptor blocker phenoxybenzamine (10^{-5}M) and with guanethidine (10^{-5}M) which blocks the release of neurotransmitters such as noradrenaline, ATP, and NPY. As shown in figure 4.27, the responses to field stimulation gradually decreased during perfusion and were almost

Fig. 4.23. The top trace shows the signal obtained from the blood flow monitor in response to electrical stimulation of the saphenous nerve. A reduction in blood flow occurs during stimulation followed by dilatation. As the nerve was sectioned proximally, heart rate and blood pressure remained constant. Bottom trace shows stimulus pulse (10HZ, 10V, 1msec).

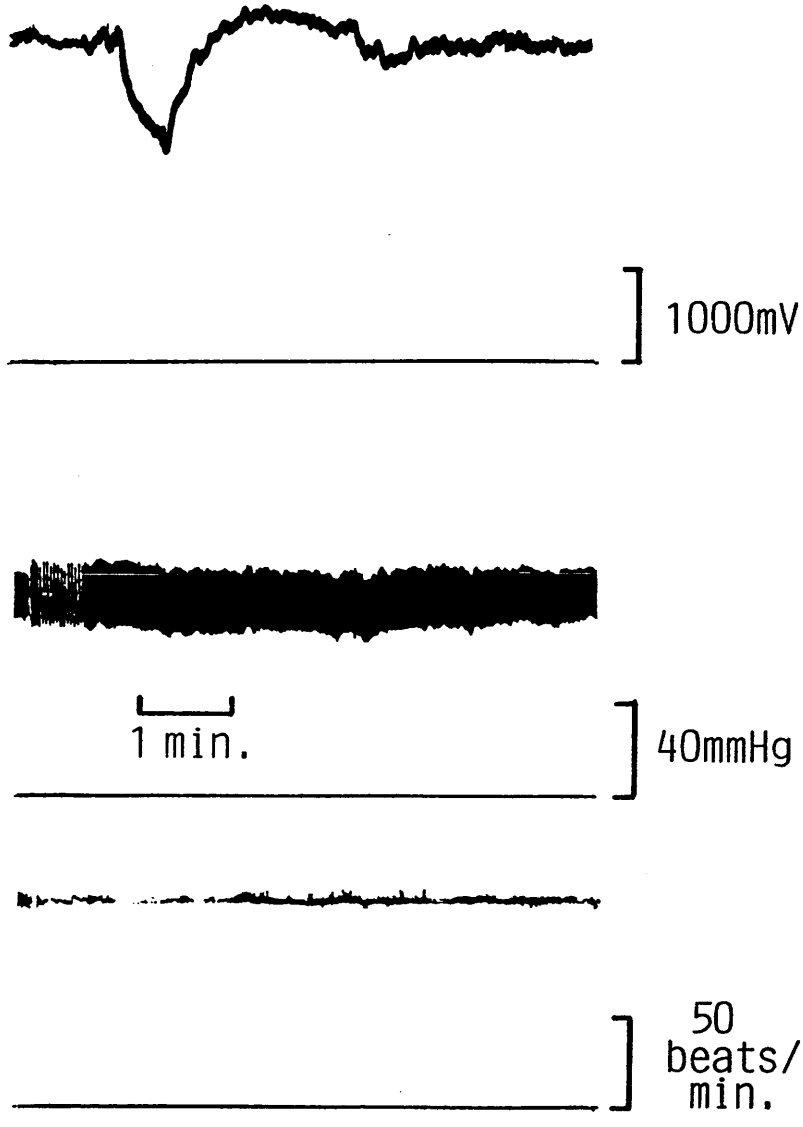

1000mV
1 min.
40mmHg
50
beats/
min.

Fig. 4.24. The effect of 100ug dose of substance P antagonist (SPA) ijected intra-articularly on the dilator response of articular blood vessels to electrical stimulation of saphenous nerve (10HZ, 10V, 1msec). The dilator response was significantly reduced by this agent. n= 7, *** P<0.001.

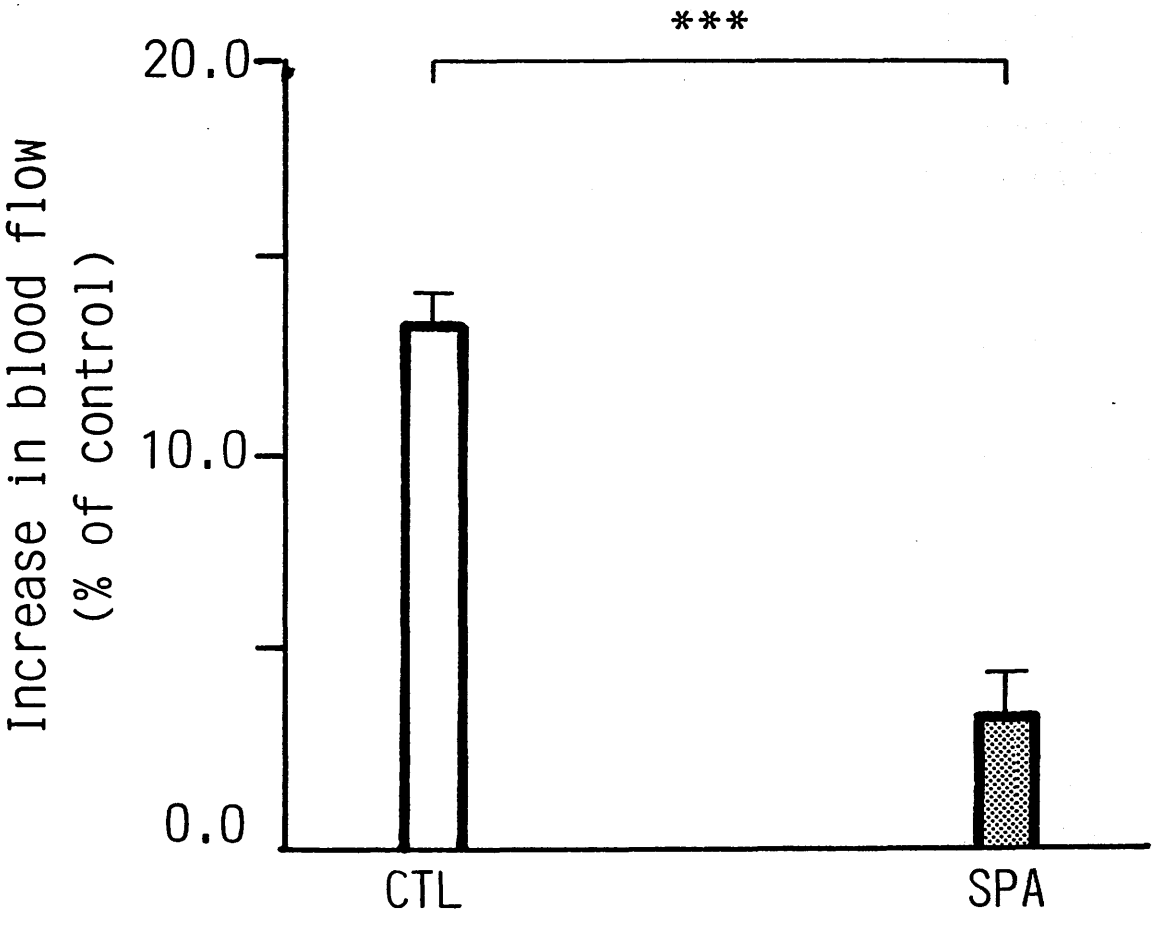

***
20.0
10.0
0.0
Increase in blood flow
(% of control)
CTL
SPA

Fig. 4.25. Trace shows the flux signal obtained from the blood flow monitor in response to electrical stimulation(s) of the saphenous nerve at different frequencies (10,20,30, 50HZ) but with the same voltage (10V) and pulse width (1msec). Arrows show where the stimulus train started (30sec duration).

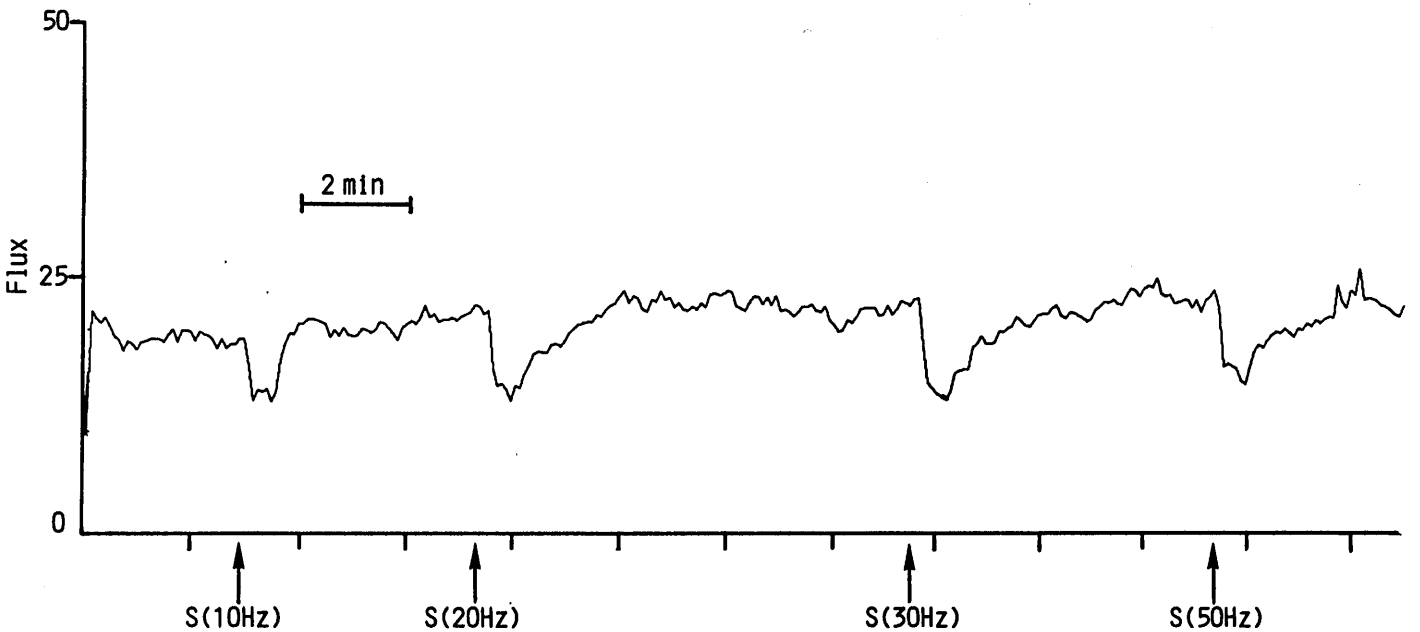

50
25
0
Flux
2 min
S(10Hz)
S(20Hz)
S(30Hz)
S(50Hz)

Fig. 4.26. Articular blood flow changes in response to saphenous nerve stimulation at different frequencies but constant voltage (10V), and pulse width (1msec). n= 5-13, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

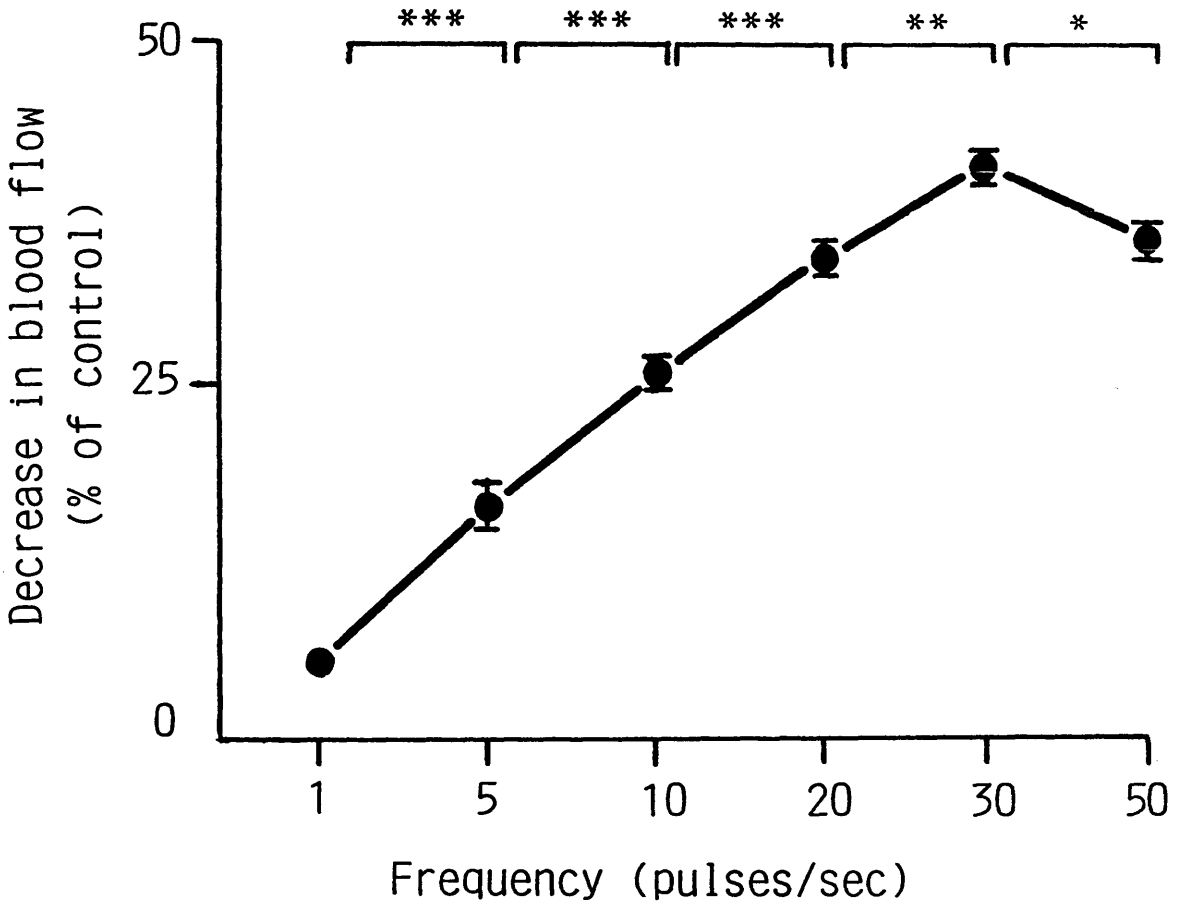
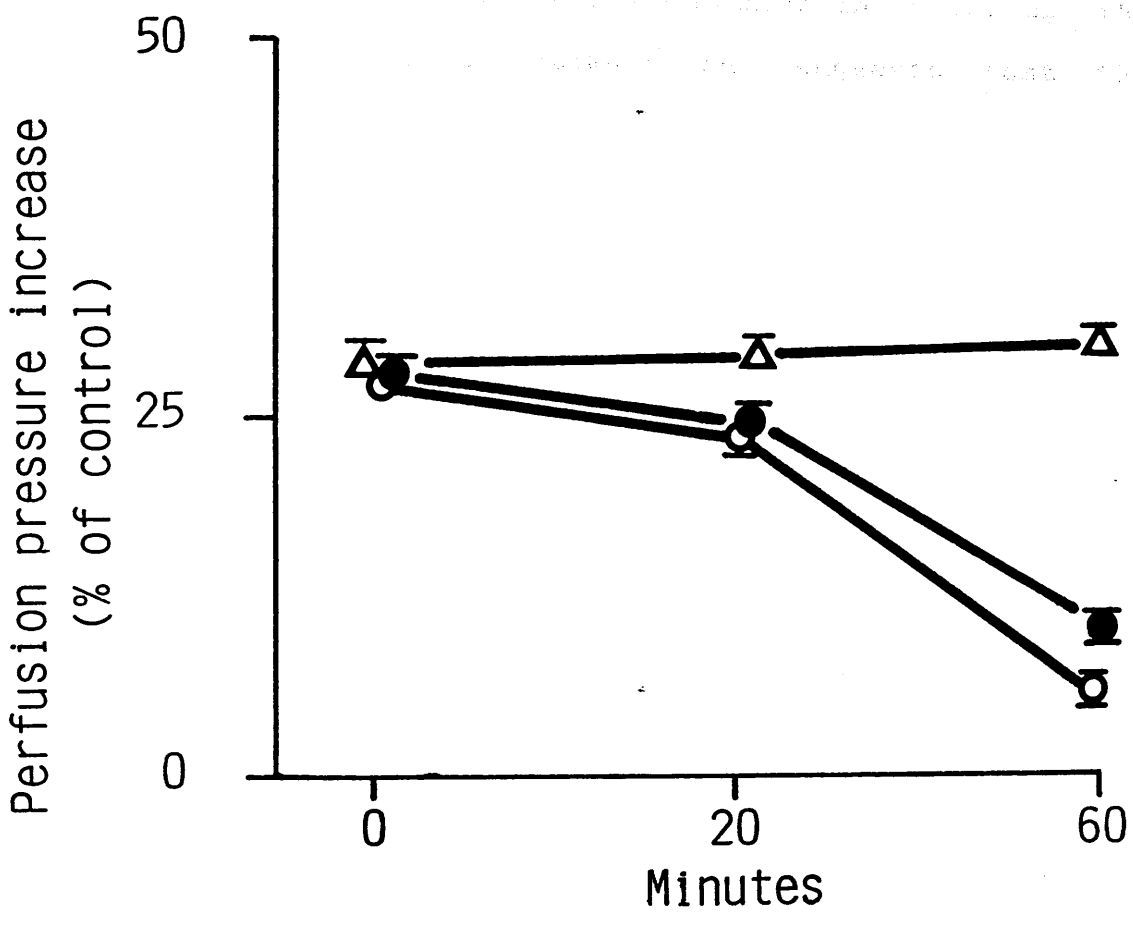

***
***
***
**
*
50
25
0
Decrease in blood flow
(% of control)
1
5
10
20
30
50
Frequency (pulses/sec)

Fig. 4.27. Comparison of the effect of continuous perfusion of the knee joint with plain Locke's solution (▲)Locke solution containing phenoxybenzamine at 10^{-5}M (O) or guanethidine at 10^{-5}M (●) on the constrictor response to field stimulation. CTL represents the response to field stimulation prior to perfusion of these agents. n = 7 - 9. ** = $p<0.01$.

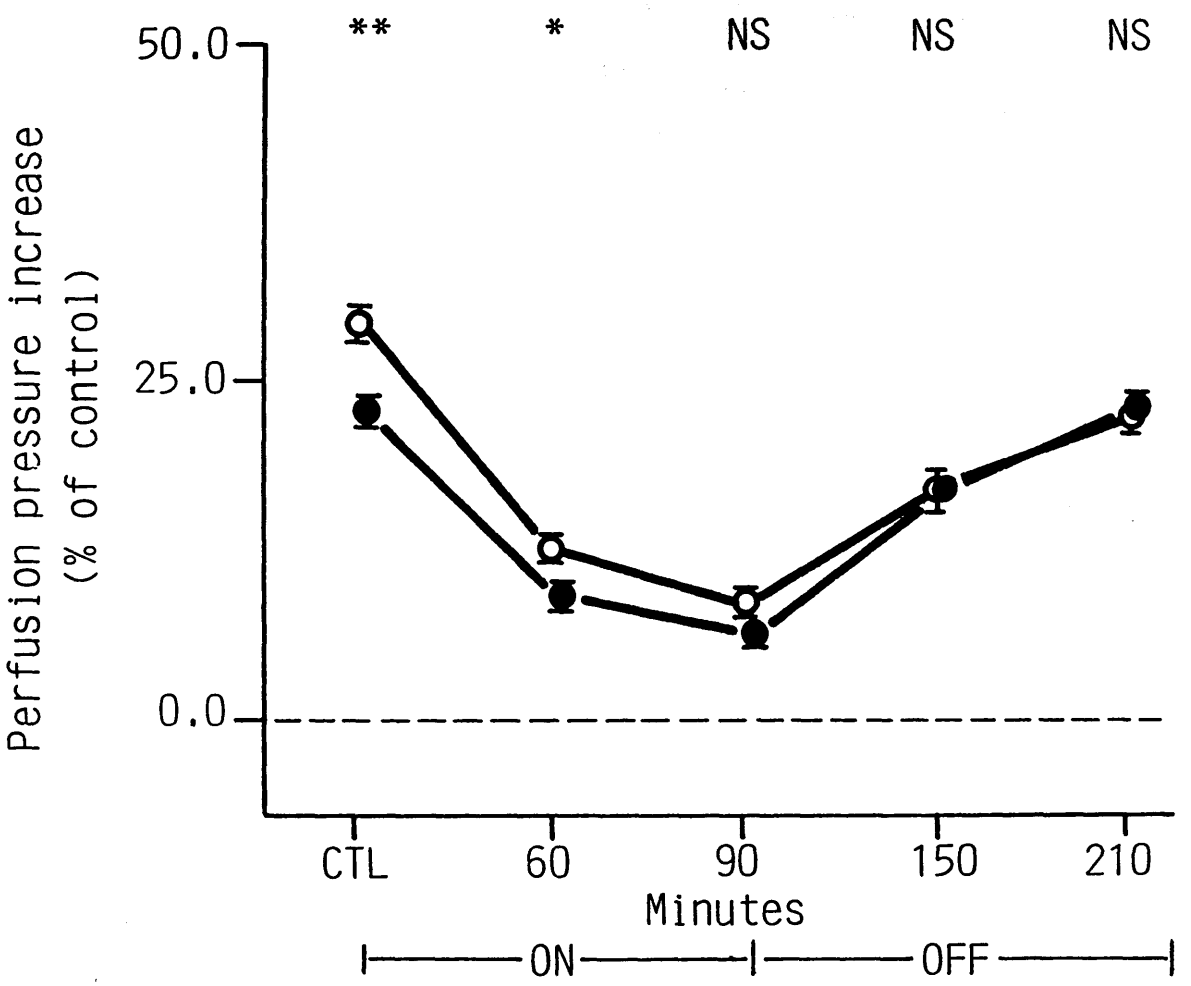

50
25
0
Perfusion pressure increase
(% of control)
0
20
60
Minutes

abolished after 60min. Both agents were almost equally effective in blocking the responses and also had similar time courses but when no antagonist was used there was no significant change in responses due to field stimulation up to 90min.

The effectiveness of phenoxybenzamine in blocking the responses to field stimulation suggests that the neurotransmitter involved may be noradrenaline acting upon α-adrenoceptors. To further investigate which type of α-adrenoceptor was involved, in other preparations the effects of the selective α_1-adrenoceptor blocker, prazosin (10^{-6}M) on the response to field stimulation was examined. As illustrated in figure 4.28, 60min after the start of perfusion with prazosin the constrictor response was substantially reduced, with a further, but smaller, decrease at 90min. On cessation of the prazosin perfusion the response gradually returned to control values by about 120min post perfusion. In another series of experiments the selective α_2-adrenoceptor blocker rauwolscine (10^{-6}M) was added to the prazosin perfusion. However, it is clear from figure 4.28 that adding rauwolscine did not significantly modify the effect of prazosin. Although there were differences in the control responses between the two groups of preparations (CTL), by 90min of perfusion there was no significant difference in the magnitude of inhibition of the constrictor response.

Intra-arterial injection of a α-blocker such as phentolamine and a selective α_1-blocker such as prazosin

Fig. 4.28. Comparison of the effect of continuous perfusion of the knee joint with prazosin at 10^{-6}M(O) and another perfusate (●) containing prazosin (10^{-6}M) plus rauwolscine (10^{-6}M). ON indicates the period during which the Locke's perfusate contained the adrenoceptor antagonists, whilst OFF indicates return to plain Locke's solution. n = 6 - 7. * = $p<0.05$; ** = $p<0.01$.


**
*
NS
NS
NS
50.0
25.0
0.0
Perfusion pressure increase
(% of control)
CTL
60
90
150
210
Minutes
ON
OFF

both attenuated the constrictor response due to saphenous nerve stimulation in the in-vivo experiments as well but not the rauwolscine an α_2-blocker(figure 4.29). These experiments suggest that the neurotransmitter which is released from sympathetic nerve ending innervating articular blood vessels is noradrenaline which acts principally via α_1-adrenoceptors.

In order to assess whether any component of the constrictor response was mediated via purinergic receptors, α , β methylene ATP (10^{-6}M), a P_2-purinoceptor desensitiser was perfused and the effect of field stimulation was examined during the period of perfusion. In none of the preparations was this agent found to influence the constrictor response even after 90 min of perfusion (figure 4.30). As illustrated in figures 4.31, 4.32, the intra-arterial injection of 0.2ml α, β mATP also did not change the response to direct saphenous nerve stimulation even when the maximum frequency (30 pulses per second) was used.

d: <u>The effect of UK-14304 on the responses to saphenous nerve stimulation</u>

Intra-arterial injection of UK-14304 a selective α_2 agonist attenuated the constrictor response due to direct saphenous nerve stimulation (Figure 4.33). This response was dose dependent with increasing concentration of UK-14304 leading to decreasing response to nerve

Fig. 4.29. The effect of close intra-arterial injection of different antagonists on the constrictor response to saphenous nerve stimulation in the rabbit. PHA; phentolamine (10^{-5}M), PRA; prazosin (10^{-5}M), RAW; rauwolscine (10^{-5}M), α, β mATP (10^{-5}M). The black histograms represent control responses before antagonists administration. n= 5-7, NS: Non significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

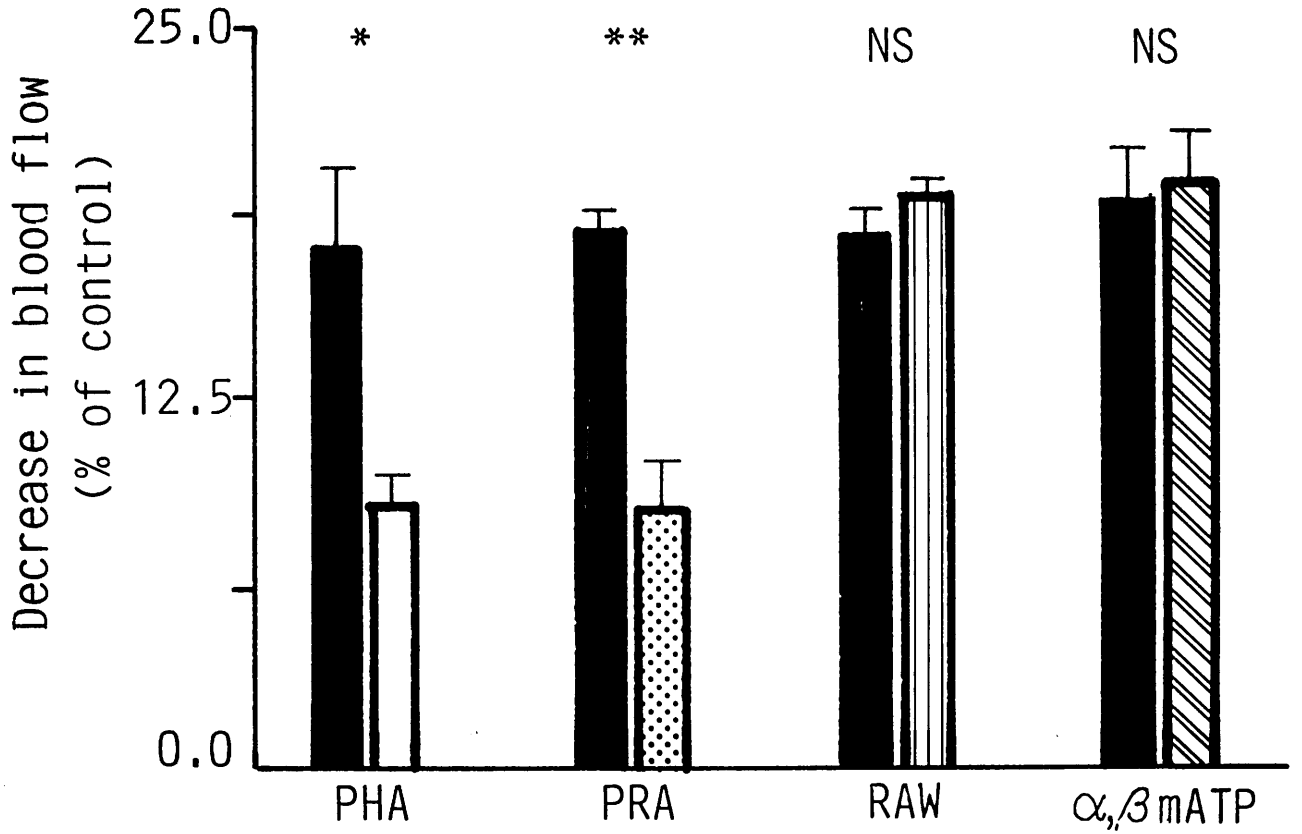

Decrease in blood flow
(% of control)
25.0
12.5
0.0
*
**
NS
NS
PHA
PRA
RAW
α,β mATP

Fig. 4.30. Perfusion of the knee joint with α,β methylene ATP (10^{-6}M) had no effect on the constrictor response to field stimulation of the joint capsule. n = 7 - 8.

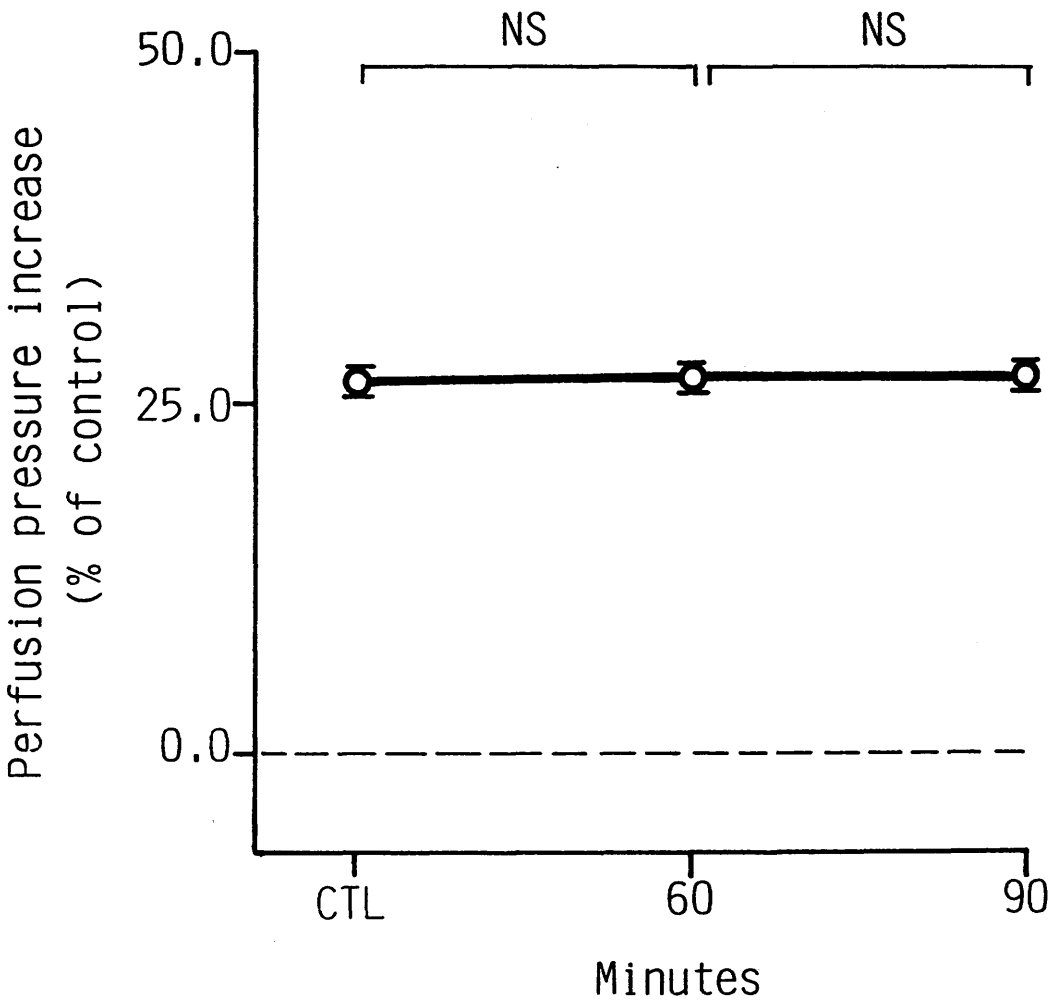

NS
NS
50.0
25.0
0.0
Perfusion pressure increase
(% of control)
CTL
60
90
Minutes

Fig. 4.31. Trace shows the effect of α, β mATP (10^{-4}M) injection on the magnitude of constrictor response in knee joint blood vessels due to saphenous nerve stimulation. α, β mATP itself caused vasoconstriction via P_2-purinoceptors (I_1), but by the second injection (I_2) these postsynaptic receptors became desensitized and there was no response by the third injection (I_3). Arrows S shows the onset of the electrical stimulus train (10V, 30HZ, 1msec, 30sec).

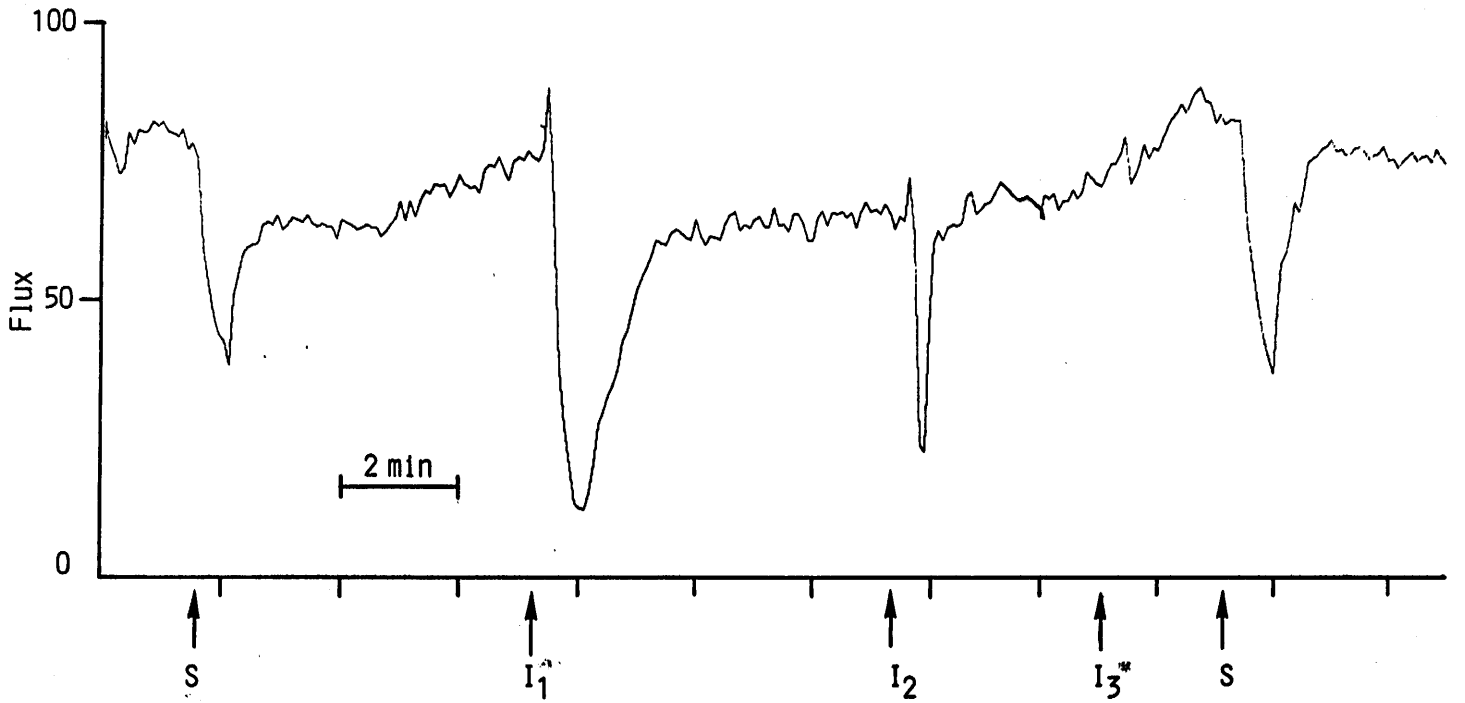

100
50
0
Flux
2 min
S
I1*
I2
I3*
S

Fig. 4.32. Comparison between the constrictor response in articular blood vessels due to saphenous nerve stimulation (10V, 30HZ, 1msec) before(CTL) and after α, β mATP. No significant changes were observed. n= 5, NS: Non significant.

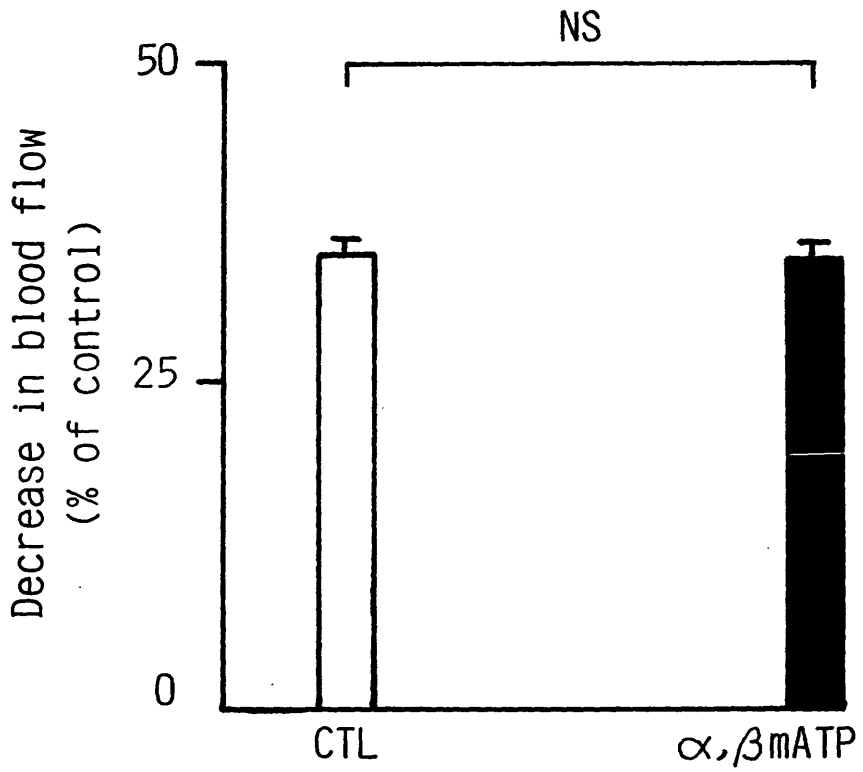
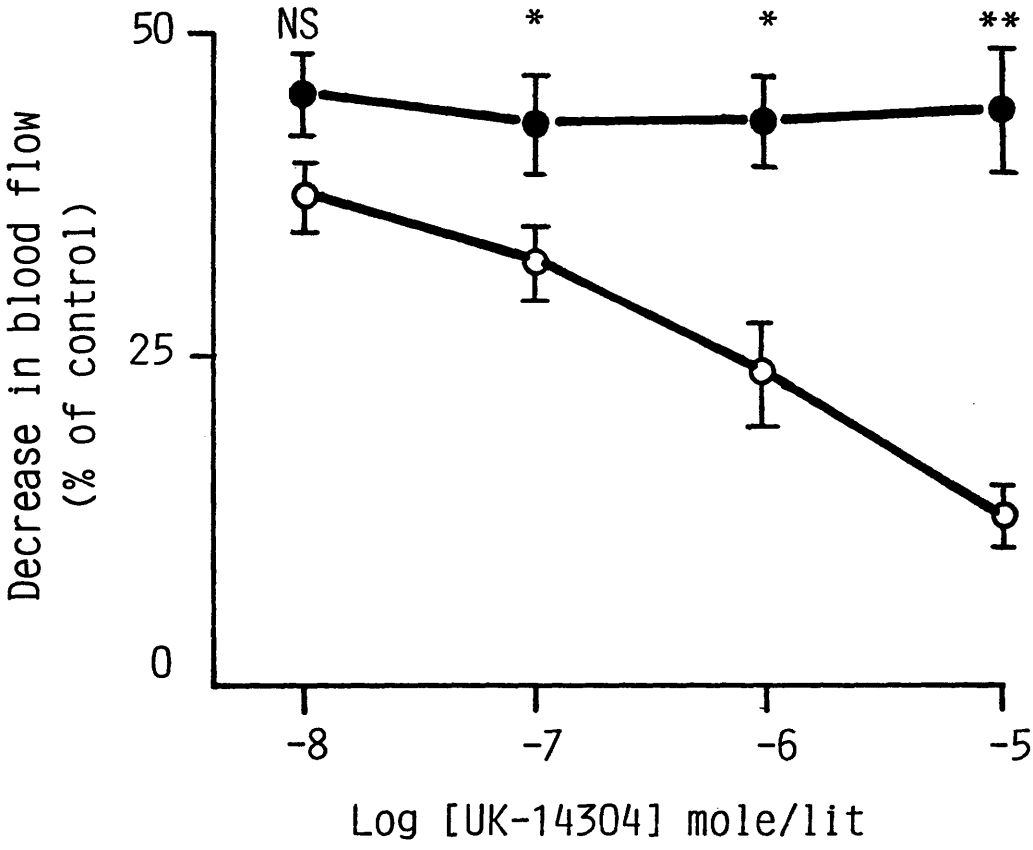

NS
50
25
0
Decrease in blood flow
(% of control)
CTL
α,βmATP

Fig. 4.33. The effect of UK-14304 on constriction of articular blood vessels in response to saphenous nerve stimulation. (●) shows the constrictor response before UK-14304 injection. (O) represents the response after the injection of UK-14304. * $P<0.05$, ** $P<0.01$. n= 8.


50
25
0
NS
*
*
**
-8
-7
-6
-5
Decrease in blood flow
(% of control)
Log [UK-14304] mole/lit

stimulation. This effect is presumly the result of activation of pre-synaptic α_2-adrenoceptors by this α_2-agonist.

DISCUSSION

The results of the present experiments demonstrate that the vasoconstrictor response to electrical stimulation of the dorsal aspect of the joint capsule is neurally mediated as it was significantly reduced by tetrodotoxin or virtually absent in animals pretreated with reserpine. Additional supporting evidence is that perfusion with the α -blocker phenoxybenzamine for about an hour could completely abolish the response to electrical stimulation. Haloalkylamines such as phenoxybenzamine are known to have a slow onset of action (at least 1/2 hour) even on systemic administration due to the time required for formation of reactive intermediates which bind with receptors (Wiener 1985). However in the in-vivo experiments close intra-arterial injection of substances such as phentolamine could attenuate the response due to nerve stimulation in short time (a few minutes). None of the above procedures would have been expected to prove successful if the vasoconstrictor response resulted from direct electrical stimulation of vascular smooth muscle.

The vasoconstrictor response to electrical stimulation was blocked almost equally well by the α -blocker phenoxybenzamine and by guanethidine which inhibits release of various transmitters, suggesting this response is principally mediated by noradrenaline. This

has also been proved by intra-arterial injection of phentolamine which attenuated the response to saphenous nerve stimulation in-vivo. If any other neurotransmitters are co-localised within sympathetic efferent fibres innervating knee joint blood vessels, these do not appear to contribute significantly to the constrictor response with the stimulus parameters employed. Noradrenaline appears to be acting upon α_1-adrenoceptors as vasoconstriction was blocked by the α_1 antagonist prazosin, but there was either no effect or little additional effect either on the magnitude or the time course by using the α_2 antagonist rauwolscine.

These results are consistent with previous observations that α -adrenoceptors are present on rabbit knee joint blood vessels and that vasoconstriction elicited by noradrenaline injection is mediated principally via α_1 -adrenoceptors (Ferrell and Khoshbaten 1989a).

The dilator response which followed after the initial vasoconstriction was substantially attenuated after injection of substance P antagonist. This suggests that, consistent with the finding of Ferrell and Cant (1987), this response is likely to be mediated by nerves which release Substance P from their terminals when depolarised.

Administration of UK-14304 (selective α_2-agonist) attenuates the reduction of articular blood flow due to saphenous nerve stimulation. This finding suggests that,

there might be prejunctional α_2 receptors which mediate inhibition of transmitter release from sympathetic nerve endings.

Although it has been shown in section I of this chapter that P_1 and P_2 purinoceptors are present within articular blood vessels, and that the P_2 receptor mediates vasoconstriction, in the present experiments no evidence was obtained to indicate that ATP is released from nerve endings and contributes towards the vasoconstriction elicited by either electrical stimulation of the joint capsule or direct saphenous nerve stimulation. The role of purinoceptors in regulating articular blood vessel calibre remains unclear at present.

In summary, all of the data is now consistent with the hypothesis that noradrenaline is the main neurotransmitter at articular blood vessels which responsible for the vasoconstrictor response. It is also suggested that the widespread sympathetic vasopressor response to stimulation of the sympathetic outflow in articular blood vessels of the rabbit comprises mainly α_1-adrenergic elements since it can be almost blocked by α_1-adrenoceptor antagonists such as prazosin but not the α_2-adrenoceptor blocker, rauwolscine.

****

*****

*

SECTION III

RESPONSES OF ARTICULAR BLOOD VESSELS TO ACUTE JOINT INFLAMMATION IN THE RABBIT

SUMMARY

1.Utilising laser Doppler flowmetry, this study has examined the responses of blood vessels in the rabbit knee joint capsule to induction of acute joint inflammation by intra-articular injection of 4% kaolin suspension.

2. Kaolin injection produced an inflammatory response which became evident about 4 hours after injection.

3. Electrical stimulation of the nerve supply to the knee joint prior to induction of inflammation produced a biphasic response - an initial vasoconstriction during the stimulation phase followed by dilatation after cessation of stimulation.

4. These responses were followed over an eight hour period and it was observed that the constrictor response became progressively greater, producing a further 19% decrease in blood flow during nerve stimulation about three hours after the injection of kaolin.

5. The sensitivity of post-junctional α -adrenoceptors showed still greater increase in the inflamed joint as close intra-arterial injection of 10^{-6}M adrenaline produced an additional 30% reduction in blood flow four hours after kaolin injection compared to the

control response.

6. Comparing above results it is possible that the lesser enhancement of the constrictor response to nerve stimulation in the inflamed joint may reflect sensitisation of pre-junctional α_2 -adrenoceptors in addition to the effects exerted on post-junctional α-adrenoceptors by the inflammatory process.

7. The dilator response was also found to increase over eight hours, although this rise was less marked.

8. These findings indicate that even over the limited time span of the experiments, significant alterations occurred in factors which can influence the calibre of articular blood vessels.

9. The functional significance of this finding is at present unclear, but it suggests that some of the mechanisms which normally regulate articular blood flow are significantly disturbed by joint inflammation.

INTRODUCTION

The factors regulating the calibre of articular blood vessels are at present poorly understood. The presence of nerves in the tissues lining diarthrodial joints has been demonstrated both by functional (Kellgren and Samuel 1950) and by histological techniques (Gardner 1950, Skoglund 1956). It has been shown that the blood vessels supplying the knee joint of the dog are innervated by sympathetic efferent fibres which when stimulated produce vasoconstriction (Cobbold and Lewis 1956b). More recently it was demonstrated that electrical stimulation of the posterior articular nerve supplying the dorsal aspect of the cat knee joint produced a biphasic response - an initial vasoconstriction during stimulation followed by a long-lasting dilatation (Ferrell and Cant 1987). The dilator response appeared to be mediated by substance P release from unmyelinated joint afferent fibres as it was inhibited by intra-articular injection of the potent substance P antagonist D-pro ^{4} D-trp ^{7,9,10} SP (4-11) (Ferrell and Cant 1987). The constrictor response is likely to be mediated via α_1 adrenoceptors as these are the principal type of postjunctional receptor found on rabbit articular blood vessels (Ferrell and Khoshbaten 1989a). Adrenaline and noradrenaline were found to be equally effective in eliciting vasoconstriction in these vessels which also appear to be devoid of β adrenoceptors (Ferrell and Khoshbaten 1989a). There would appear,

however, to be little information available about the adrenergic control of normal and diseased synovial tissue.

Rheumatoid arthritis is a common clinical syndrome characterised by a chronic symmetric inflammation with a predilection for the more peripheral joints, which eventually results in joint destruction. 0.3-1.5% of the population in the world are affected by joint inflammation (Fitzgerald 1989). Among animal model it was reported that joint pain itself may directly result in the observed morbidity, so its treatment may have significant benefits beyond alleviation of suffering (Dardick et al 1986, Landis et al 1988).

Pearson and Wood (1959) have concentrated on the role of the nervous system in arthritis. Their study was possible because of existence of suitable animal models for arthritis which in their clinical manifestation mimic those of rheumatoid arthritis. Works on the effect of the nervous system in arthritis were limited to some clinical signs, such as chronic pain, reduced activity, having fragmented sleep patterns, hyperventilation, increased sensitivity to paw pressure or flexion and extension of inflamed joints (Calvino et al 1987,Landis et al 1988).

Calvino and his colleagues (1987) concluded that, in arthritic rats, the sensitivity to light pressure on the inflamed skin and gentle movement of the affected joint are increased.

Levine and his colleagues have recently also produced evidence that the peripheral limb of the sympathetic nervous system is involved in arthritis. They found that sympathectomy markedly reduced inflammation and joint injury in the experimental arthritic model (Levine et al 1986). In another experiment, rats treated with β_2-adrenergic antagonists showed significantly reduced joint injury (Levine et al 1988).

The present experiments were performed to examine whether the response to electrical stimulation of the nerve supply (sympathetic efferent and afferent C fibres) to the rabbit knee joint is altered by experimentally induced acute joint inflammation. This was attempted to establish whether the vasodilation known to occur in joint inflammation could be attributed to alteration of neurotransmitter release at sympathetic nerve endings or change in adrenoceptor sensitivity induced by the inflammatory process.

EXPERIMENTAL PROTOCOL

Adult albino New Zealand rabbits weighing about 3-3.5kg were anaesthetised with a mixture of diazepam (0.5mg/kg IP) and hypnorm (0.1mg/kg IM). Thereafter, anaesthesia was maintained using a gaseous mixture of O_2/N_2O/halothane (1-2%) which was delivered via a tracheal cannula. The rest of surgical procedures were carried out as described previously (Chapter two, in-vivo preparation). The antero-medial aspect of the knee was exposed and the patellar ligament sectioned close to the patella and the underlying articular tissue carefully exposed. A 21G hypodermic thermocouple was inserted into the joint cavity and connected to an amplifier (Thermalert TH-6D) to provide a measure of intra-articular temperature. Relative changes in blood flow were measured using a near infra-red (780nm) laser Doppler flowmeter (Moor Instruments MBF2). The laser Doppler technique has been shown previously to provide a means of assessing synovial blood flow in the human knee (Geboreck et al 1989). The laser probe was placed on the surface of the capsule as defined in chapter 2 and the surrounding tissue covered with cling film. The signal from the flowmeter was recorded on one channel of a pen recorder with blood pressure being recorded on the other channel.

The saphenous artery was cannulated as described in section II of this chapter. The saphenous nerve was

dissected free close to the groin, the proximal end cut and all branches except those to the knee were sectioned. Bipolar silver wire electrodes were then placed on the distal end of the nerve and the stimulator (Harvard Advanced stimulator) was set to deliver 30sec trains of rectangular pulses of 1msec width, 10 Hz frequency, and 10V amplitude.

Acute inflammation was induced by intra-articular injection of 4% kaolin suspension (Sigma) into one knee joint with the other being injected with sterile physiological saline to act as an internal control. The responses of the two knees were examined by monitoring intra-articular temperature and by measurement of Evans blue extravasation into the joint tissue of the two knees. As Evans blue binds to plasma proteins normally restricted to the vascular compartment, its presence in joint tissues provides an indirect means of assessing capillary permeability. The technique used to extract Evans blue and its subsequent spectrophotometric measurement has been described previously (Lam and Ferrell 1989). Evans blue was injected intravenously in a dose of 75mg/kg prior to intra-articular injection and the tissue samples were obtained at the conclusion of the experimental period (~8 hr after intra-articular injection of saline or kaolin).

RESULTS

a: Saphenous nerve stimulation

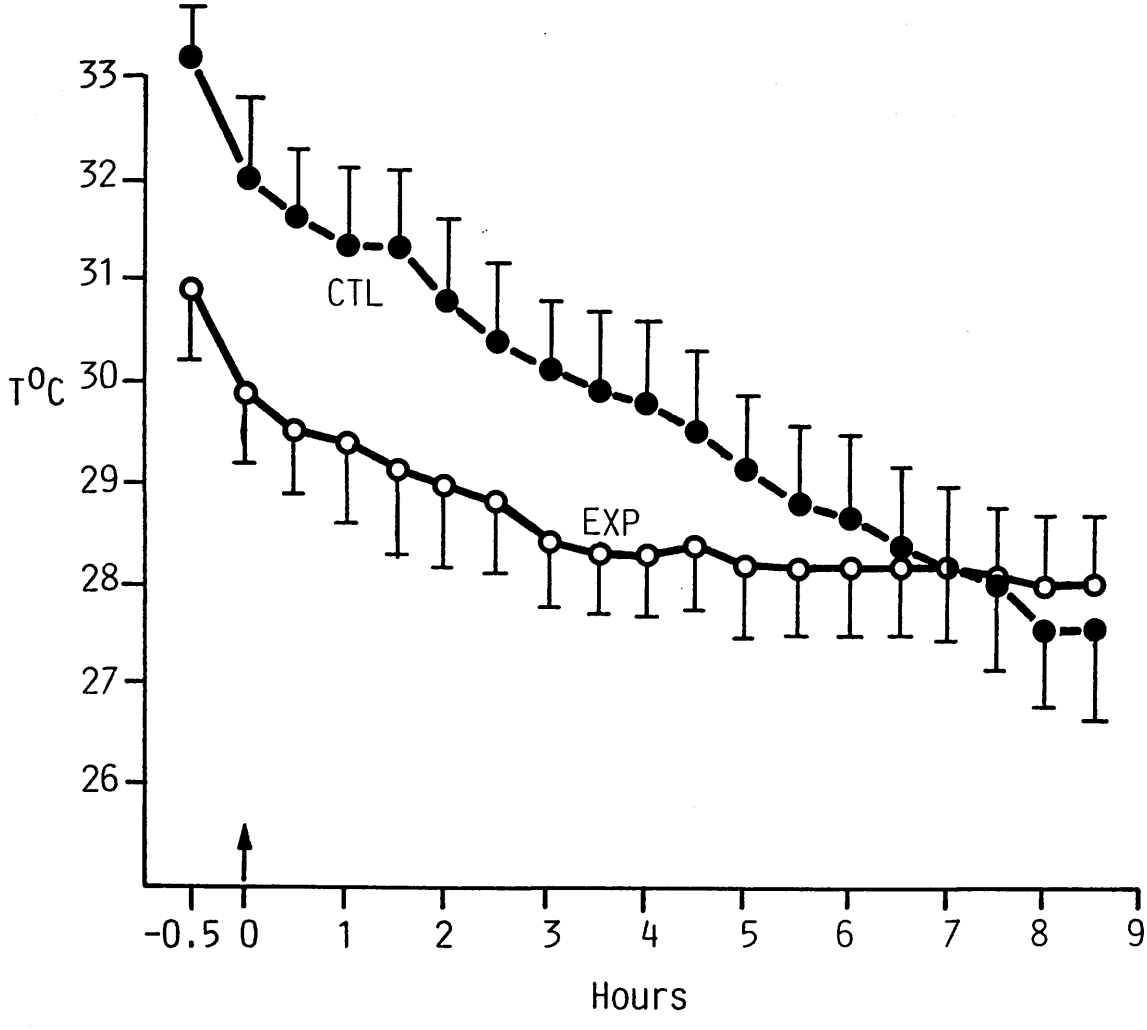
Electrical stimulation of the saphenous nerve produced a repeatable response consisting of reduction in blood flow during the period of nerve stimulation, indicating vasoconstriction, followed by a longer-lasting dilator response (Figure 4.23).

This response to nerve stimulation remained relatively stable and reproducible upon repeated stimulation, as long as the 30sec pulse trains were not re-applied until 5min had elapsed from the previous period of stimulation.

b: Temperature monitoring of the knee joint cavity

Simultaneous monitoring of the intra-articular temperatures of the two knees from the same animal revealed characteristic responses. The knee which was injected with saline showed a progressive fall in intra-articular temperature with time (Figure 4.34), presumably due to cooling of the immobile knee. The knee injected with kaolin also showed a fall in temperature initially, but this eventually levelled out (Figure 4.34). The 2°C difference in the temperatures before injection was due to the kaolin- injected knee being exposed to the atmosphere, in order to obtain the blood flow

Fig. 4.34. Change in intra-articular temperature in knees injected with saline (●) compared to those injected with 4% kaolin suspension (O). The injections were administered at the arrow. Means ± SEM; n = 5 in both cases.


33
32
31
30
29
28
27
26
T°C
CTL
EXP
-0.5
0
1
2
3
4
5
6
7
8
9
Hours

measurements. It is noticeable that the initial temperature difference between the knees was almost completely reversed by the end of eight hours. This suggests that the kaolin-injected knee was becoming inflamed by this time.

c: Evans blue injection

Injection of Evans blue (75mg/kg) had no effect on articular blood flow (Figure 4.35). As shown in figure 4.36, the constrictor response to saphenous nerve stimulation did not change significantly. After eight hours, measurement of Evans blue content in the two knees of 5 rabbits revealed significant inflammation in the kaolin-treated side compared to the saline-injected side (Figure 4.37).

d: The effect of kaolin injection

The responses to nerve stimulation were monitored in two groups of animals every half hour over a period of 7-8 hours. One group consisted of animals where the knee was injected with saline (n = 3) whilst the other group (n = 5) consisted of animals where the knee was injected with the kaolin suspension. As shown in figure 4.38, prior to kaolin injection, nerve stimulation resulted in about a almost 30% reduction in blood flow. This constrictor response to nerve stimulation increased in amplitude after kaolin injection, producing a further 19% reduction of

Fig. 4.35. Comparison between the blood flow base-line before (CTL) and after injection of Evans blue,75mg/kg (EVB). There is no effect of Evans blue on the flowmeter signal. n= 5, NS: Non significant.

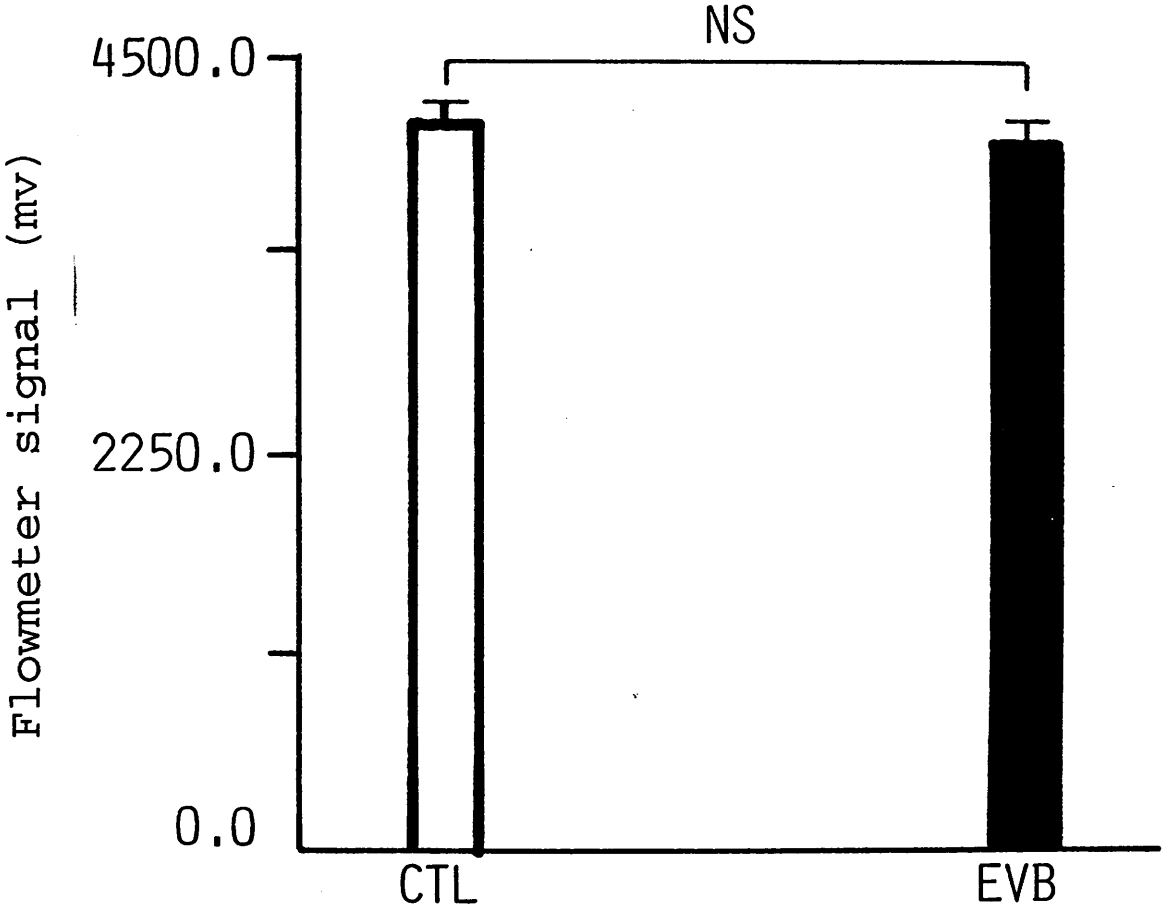

NS
4500.0
2250.0
0.0
Flowmeter signal (mv)
CTL
EVB

Fig. 4.36. The effect of Evans blue injection (75mg/kg) on the vasoconstrictor response of saphenous nerve stimulation in rabbit. No significant effect was observed after Evans blue injection. n= 5, NS: Non significant.

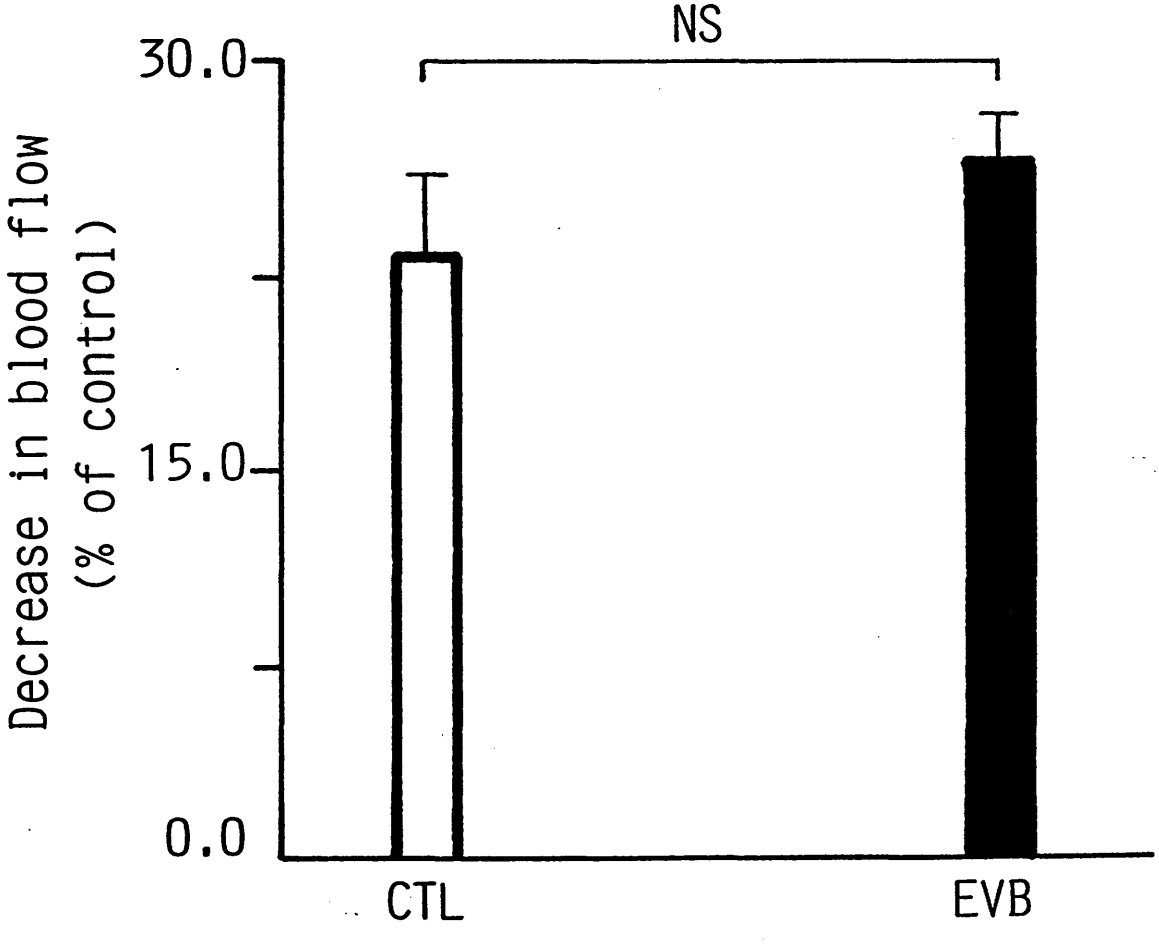

NS
30.0
15.0
0.0
Decrease in blood flow
(% of control)
CTL
EVB

Fig. 4.37. Comparison of Evans blue content of the joint capsule in saline-injected knees (filled histogram) with knees injected with 4% kaolin (open histogram). A significant difference in Evans blue content occurred in the posterior capsule (P-CAP). Although Evans blue content was also elevated in the anterior capsule (A-CAP) in the kaolin-injected knee compared to control, this was not significant. Means $\pm$ SEM; n = 5 ; * = $p<0.05$.

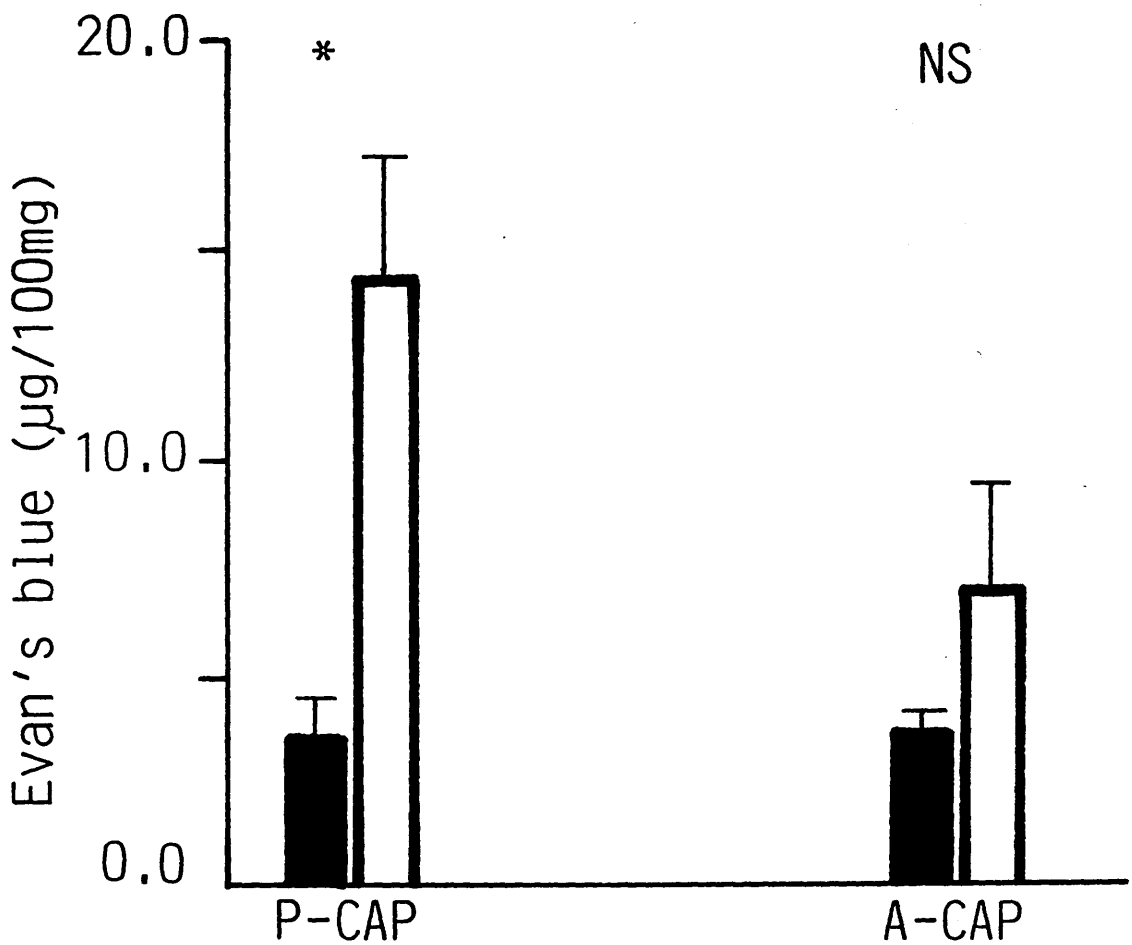
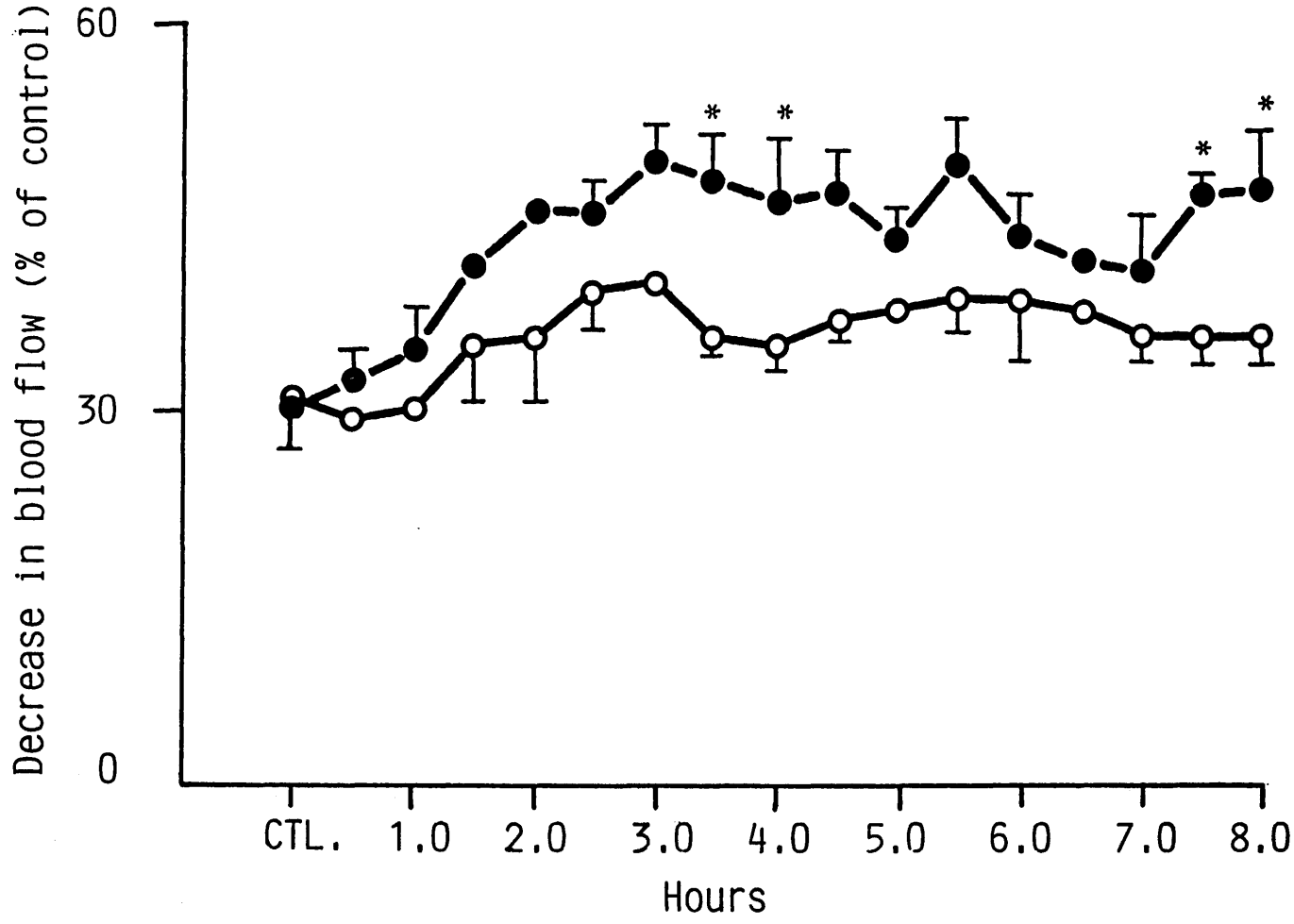

20.0
10.0
0.0
Evan's blue (μg/100mg)
*
NS
P-CAP
A-CAP

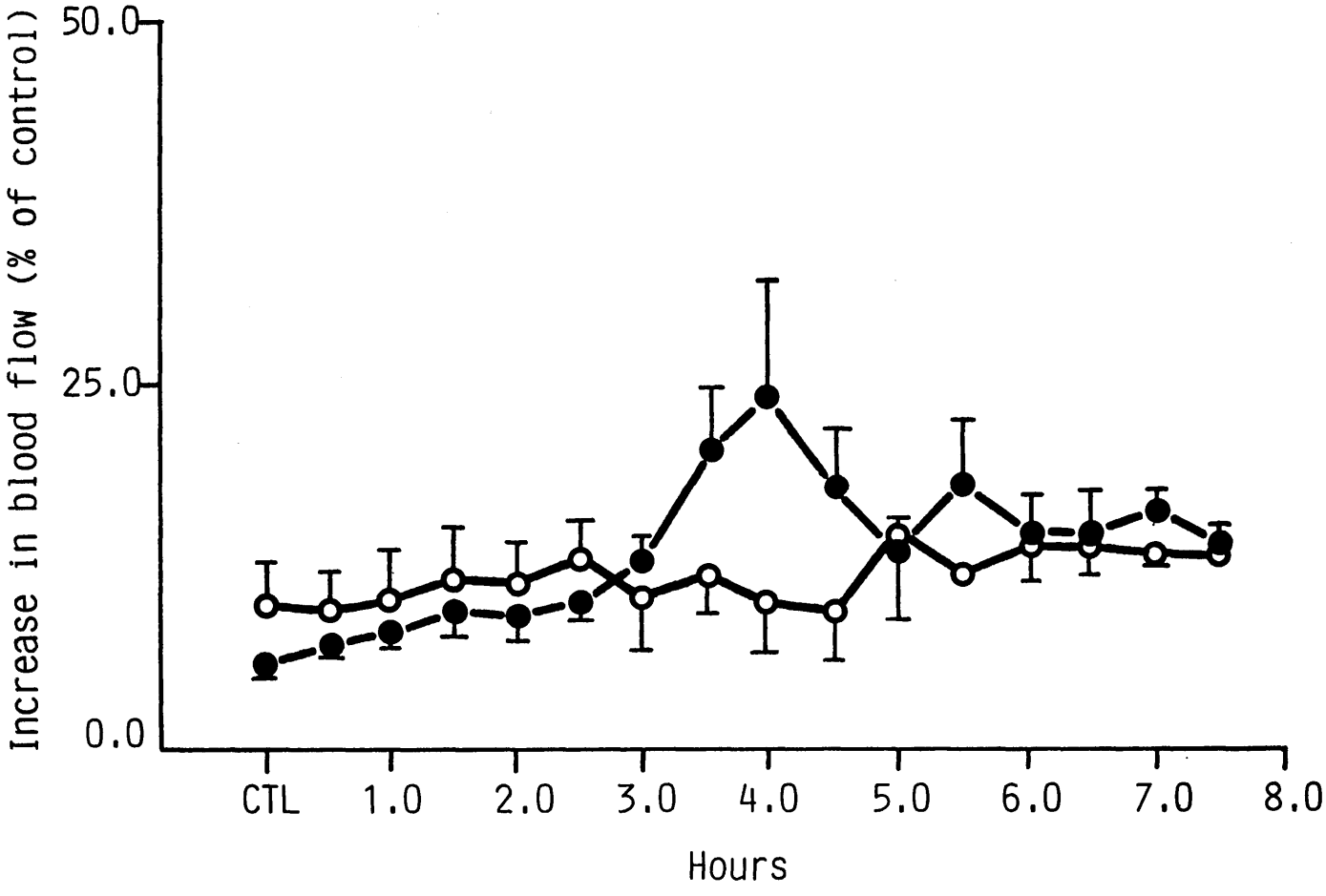
Fig. 4.38. Effect of intra-articular kaolin injection (●) and saline injection (O) on the magnitude of the constrictor response to nerve stimulation over eight hours. Change in blood flow is expressed as percentage decrease in blood flow compared to baseline value immediately before stimulation. CTL represents the response to nerve stimulation immediately prior to injection of kaolin or saline. Means ± SEM; n = 5; * = $p<0.05$.


Decrease in blood flow (% of control)
60
30
0
CTL.
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Hours
*
*
*
*

blood flow some three hours after injection which fluctuated thereafter, but always remained greater than the constrictor response in the saline-injected knees. It is noticeable that the latter also showed some enhancement, but this was of lesser magnitude (9% maximum).

The dilator response to nerve stimulation showed less obvious change with time (Figure 4.39). Although the mean value of the kaolin-injected knee peaks a four hours, this did not show a statistically significant difference from the value obtained from saline-injected knees at this time. However, comparison of the magnitude of the dilator response to nerve stimulation prior to injection of kaolin with the values obtained after injection reveals that at six hours the dilator response becomes significantly greater than control ($p<0.05$) and by seven hours the difference is greater still ($p<0.001$). In the saline-injected knees, although some variation in the dilator response occurred over the seven hour period, none of the values differed significantly from the control (pre saline injection) value. This difference may be partly attributable to the control value for the kaolin-injected knees being less than the saline-injected knees and partly due to the greater variability (as shown by the standard error bars) of the control values in the saline-injected knees compared to kaolin-injected knees. Although the means of the control values differed between the two knees, this difference was not statistically significant.

Fig. 4.39. Effect of intra-articular kaolin injection (●) and saline injection (O) on the magnitude of the dilator response following nerve stimulation. In this case the change in blood flow is expressed as the percentage increase in flow relative to the value obtained immediately prior to nerve stimulation. CTL represents the dilator response obtained prior to injection of kaolin or saline. Means $\pm$ SEM; n = 5; * = $p<0.05$.


Increase in blood flow (% of control)
50.0
25.0
0.0
CTL
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Hours

Even though the joint capsule was exposed to the external environment for many hours, blood flow (as measured by changes in flux values compared to control values) changed relatively little over this time in the saline-injected knee (Figure 4.40). In the kaolin-injected knee little change in blood flow occurred until about 6.5 hours post injection when statistically significant differences in blood flow occurred compared to the saline-injected knee. When compared to the control value, the kaolin- injected knees showed consistently significant differences at 4.5 hours and beyond whereas none of the values differed significantly from control in the saline-injected knees.

e: The effect of kaolin on adrenergic receptors

Although the responses to nerve stimulation were modestly enhanced by the inflammatory process, larger changes occurred in the sensitivity of the adrenoceptors as the response to close intra-arterial injection of adrenaline (10^{-6}M) was modified (Figure 4.41). Immediately after intra-articular injection of saline or kaolin there was little difference in the constriction induced by adrenaline which at this dose produced about a 50% reduction in flow. However, when the same dose of adrenaline was administered 2 hours later, a highly significant rise in the response occurred in kaolin-injected knees, with saline-injected knees showing

Fig. 4.40. Measurement of change in blood flow in kaolin-injected knees (●) compared to saline-injected knees (O) over seven hours. Change in blood flow is relative to the control value (CTL) obtained immediately after injection of these substances. Means ± SEM; n = 5; * = $p<0.05$.

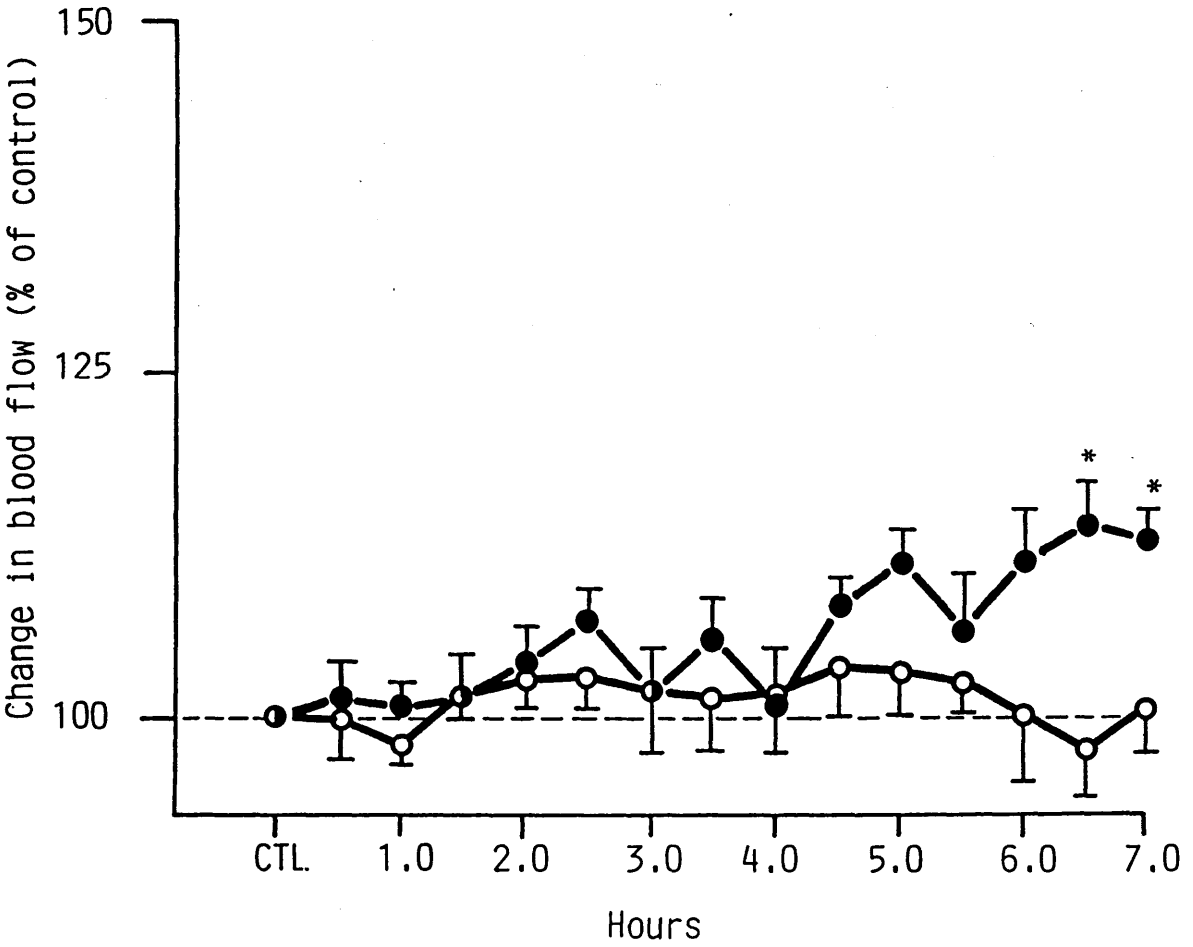
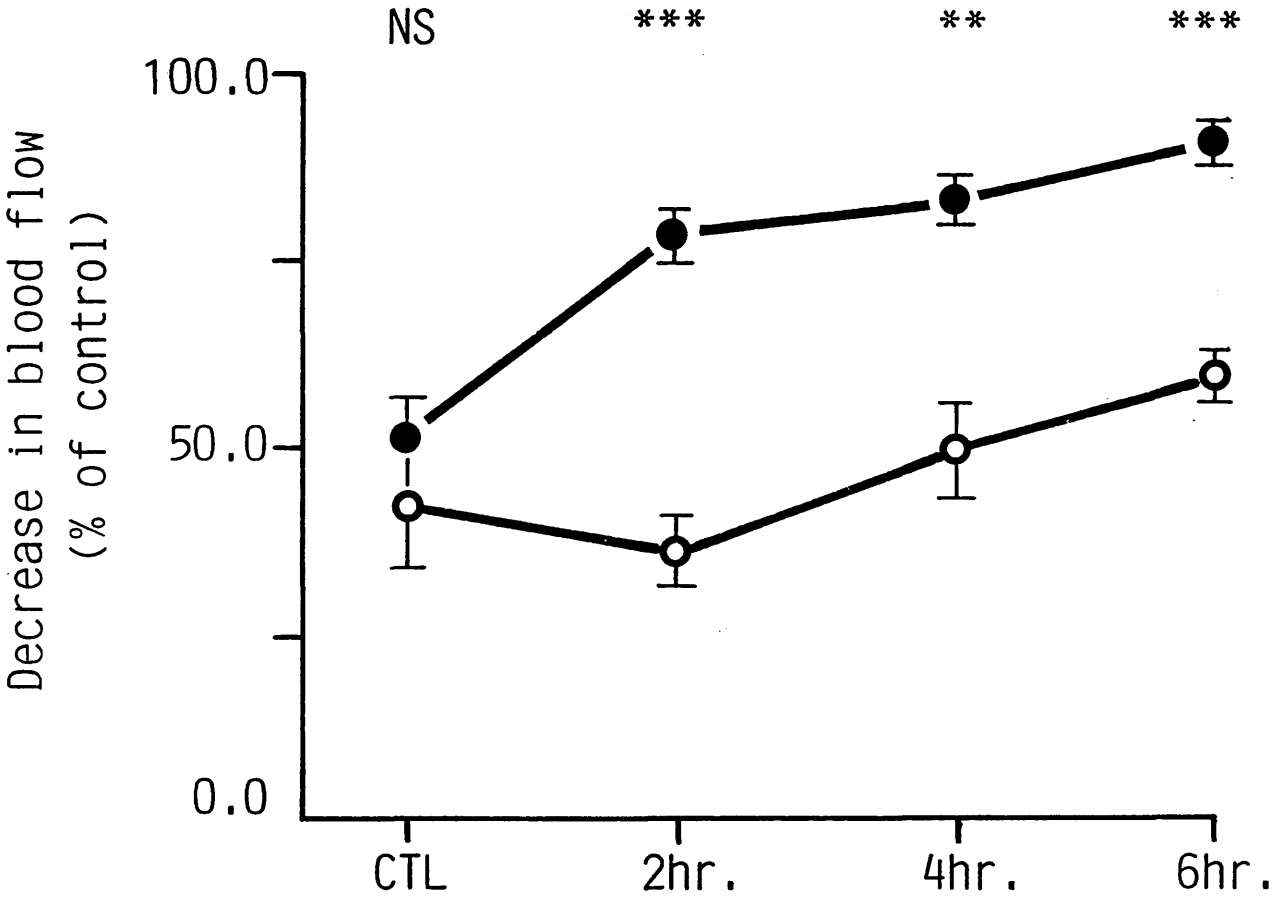

150
125
100
Change in blood flow (% of control)
*
*
CTL.
1.0
2.0
3.0
4.0
5.0
6.0
7.0
Hours

Fig. 4.41. Response to close intra-arterial injection of 10^{-6}M adrenaline in animals with kaolin-injected knees (● ; n = 6), compared to the response obtained in animals with saline-injected knees (O ; n = 3). Immediately after injection of these substances (CTL) the means do not differ significantly. After two hours the responses in the kaolin-injected knees are greater and remain elevated thereafter compared to saline-injected knees. *** = $p<0.001$; ** = $p<0.01$


NS
***
**
***
100.0
50.0
0.0
Decrease in blood flow
(% of control)
CTL
2hr.
4hr.
6hr.

a slight reduction in response. Although the saline-injected knees exhibit a trend of rising sensitivity to adrenaline with time, none of the values differ significantly from the control response, whereas in the kaolin injected knees all the responses differ significantly from control ($p<0.001$). Six hours after kaolin injection the same dose of adrenaline now produced about a 90% reduction in flow.

The differences in response to intra-arterial injection of adrenaline are well demonstrated in figure 4.42, which shows dose/response curves to adrenaline. Figure 4.43, shows that the response to adrenaline eight hours after injection of kaolin is enhanced compared to saline injection after the same length of time. Figure 4.44 is from the same animal and indicates that the response to injected adrenaline is only little affected by time.

f: The effect of cooling on adrenoceptor sensitivity.

On cooling the knee joint artificially with a fan, intra-articular temperature was reduced from 32.5 °C to 28.4 °C. Administration of 0.2ml adrenaline 10^{-6}M before and after cooling failed to show significant changes either in sensitivity nor in articular blood flow (Figures 4.45, 4.46). Thus the possibility that cooling may have increased the sensitivity was ruled out.

Fig. 4.42. A: Dose/response relationship to close intra-arterial injection of adrenaline in kaolin-injected knee (●) compared to saline-injected knee (O) eight hours after intra-articular injection of these substances.

B: Dose/response relationship to close intra-arterial injection of adrenaline in a saline-injected knee immediately after the intra-articular injection (□) and eight hours later (■) in the same animal.

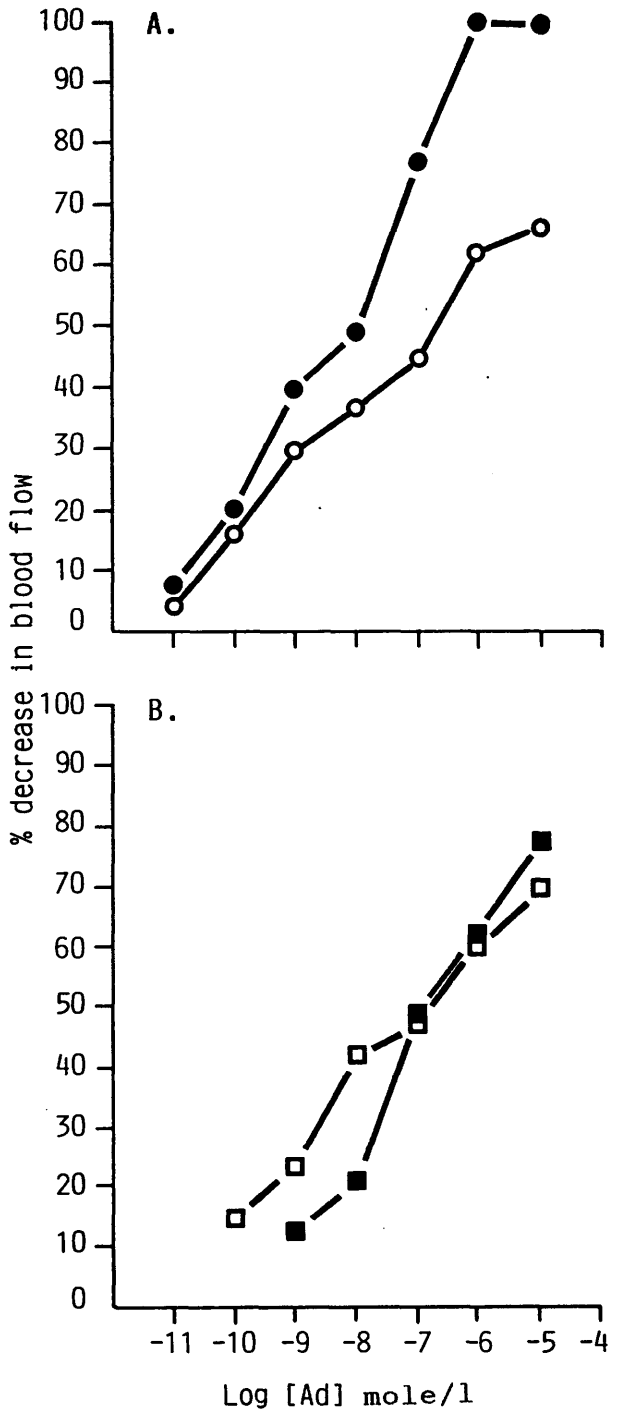

A.
B.
100
90
80
70
60
50
40
30
20
10
0
% decrease in blood flow
-11
-10
-9
-8
-7
-6
-5
-4
Log [Ad] mole/l

Fig. 4.43. Traces show dose/response relationship of articular blood vessels to close intra-arterial injection of adrenaline in the knee pretreated with kaolin after eight hours (A) and in the saline-injected knee after same period of time (B). Arterial blood pressure and the heart rate were also monitored. Dot on the time scale indicate one minute.

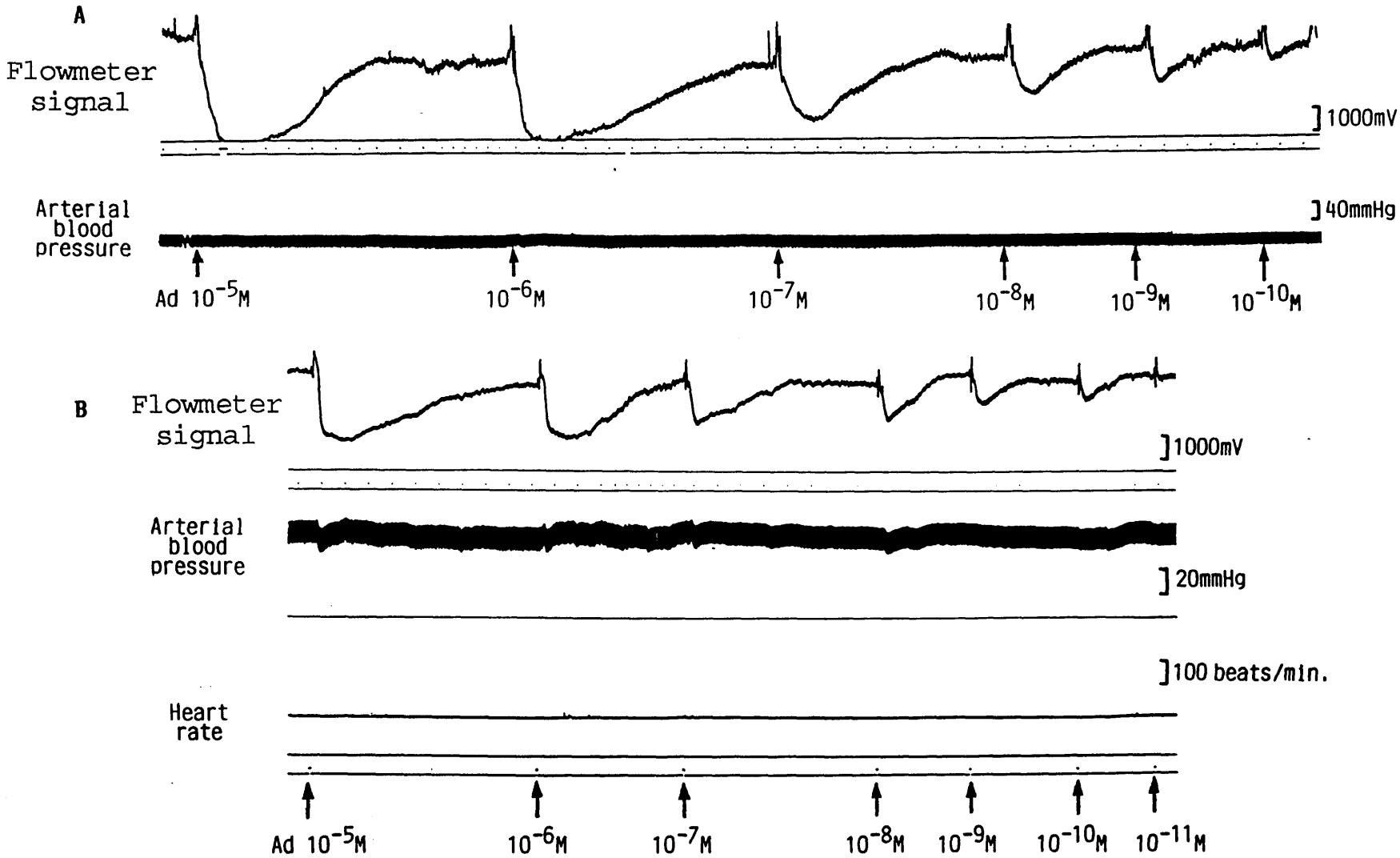

A
Flowmeter signal
1000mV
Arterial blood pressure
40mmHg
Ad 10^{-5}M
10^{-6}M
10^{-7}M
10^{-8}M
10^{-9}M
10^{-10}M
B
Flowmeter signal
1000mV
Arterial blood pressure
20mmHg
100 beats/min.
Heart rate
Ad 10^{-5}M
10^{-6}M
10^{-7}M
10^{-8}M
10^{-9}M
10^{-10}M
10^{-11}M

Fig. 4.44. Traces show dose/response relationship to close intra-arterial injection of adrenaline in the normal knee at the begining (A) and after eight hours (B) with inta-articular injection of 2ml normal saline. Dot on the time scale indicate on minute.

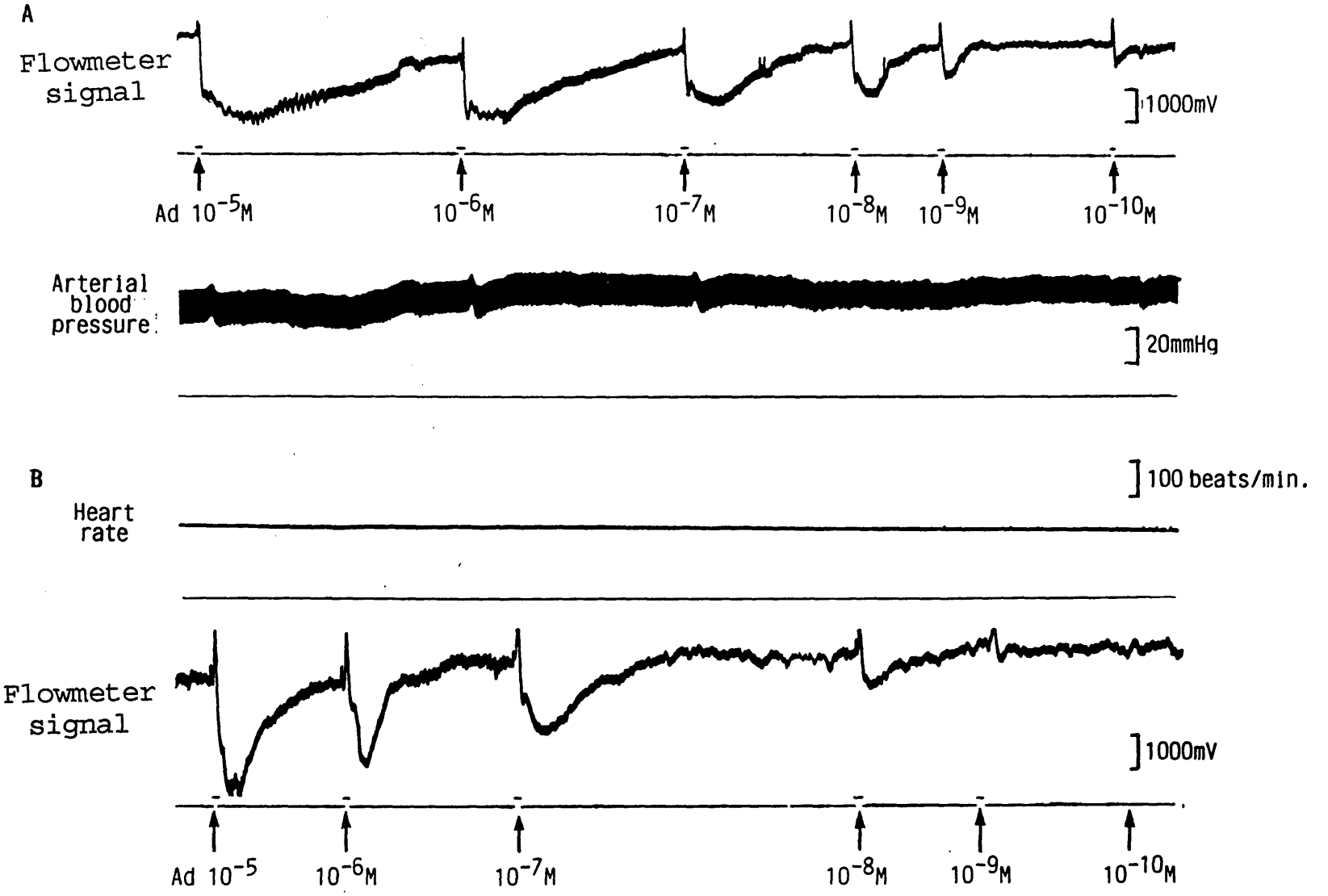
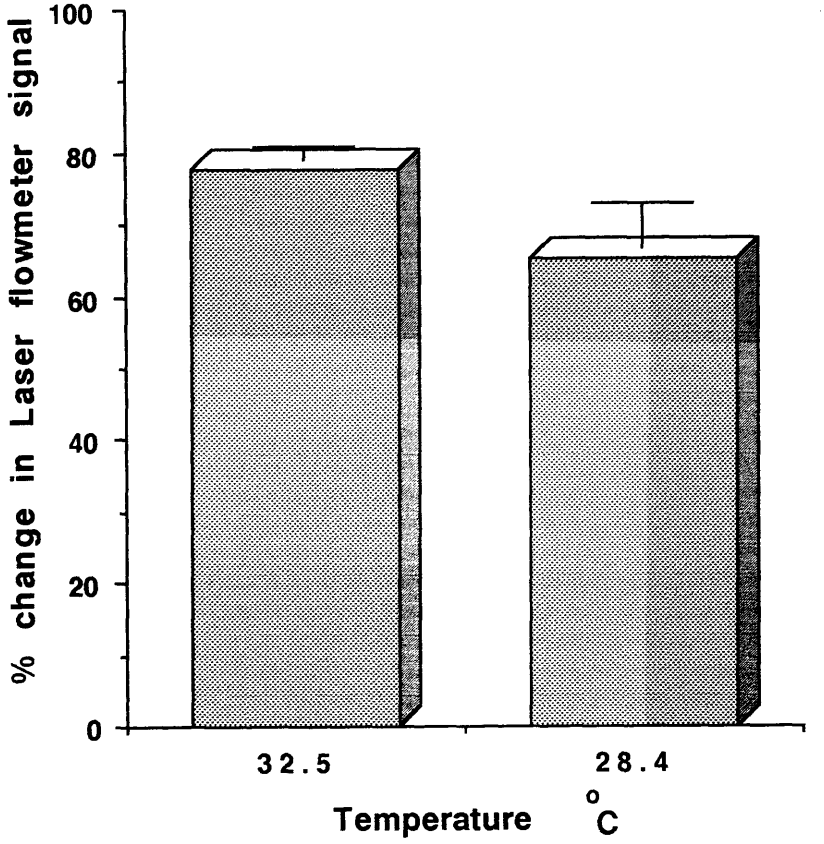

A
Flowmeter signal
1000mV
Ad 10^{-5}M
10^{-6}M
10^{-7}M
10^{-8}M
10^{-9}M
10^{-10}M
Arterial blood pressure
20mmHg
B
100 beats/min.
Heart rate
Flowmeter signal
1000mV
Ad 10^{-5}
10^{-6}M
10^{-7}M
10^{-8}M
10^{-9}M
10^{-10}M

Fig. 4.45. Upper panel: Responses of capsular blood vessels to adrenaline (10^{-6}M) injection at different temperatures (32.5 °C & 28.4 °C). No significant change was observed. n= 4.

Lower panel: The signal obtained from the blood flow monitor at different temperature (32.5 °C & 28.4 °C) show no significant changes. n= 4.


% change in Laser flowmeter signal
100
80
60
40
20
0
32.5
28.4
Temperature °C

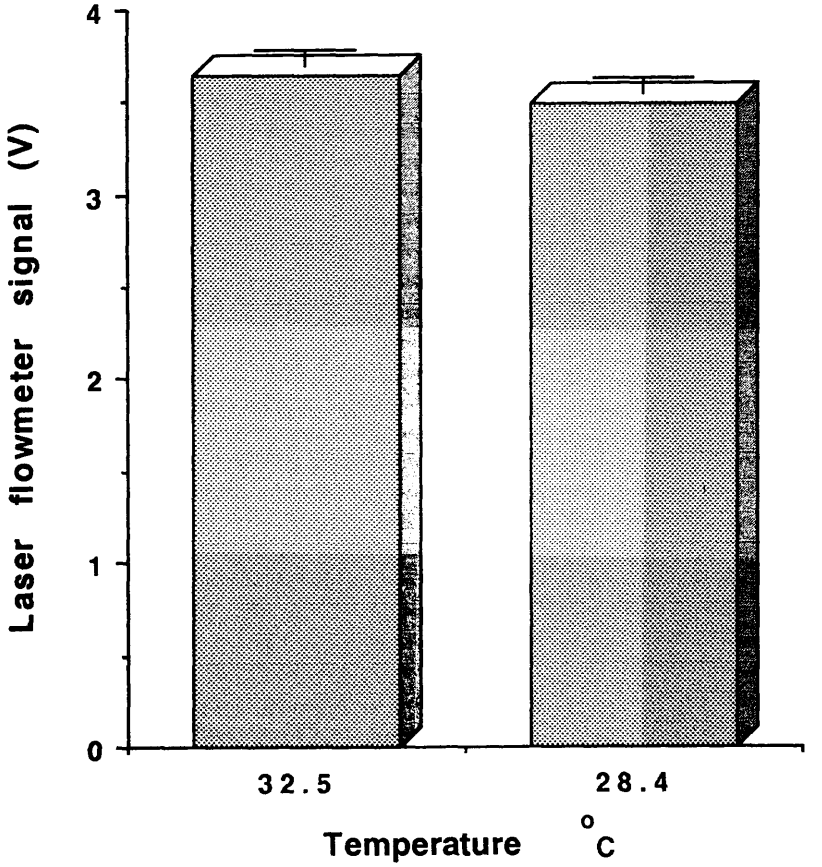
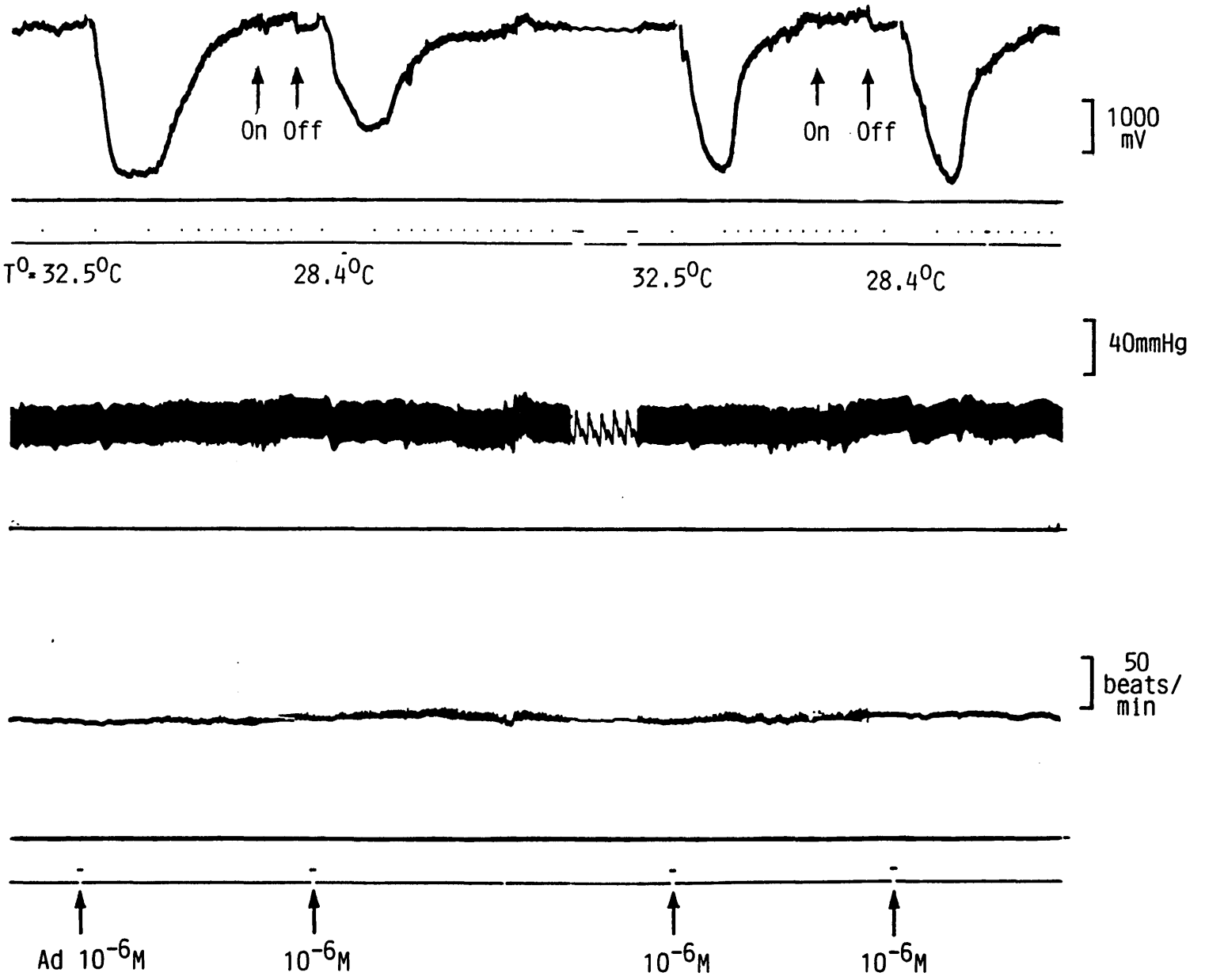

Laser flowmeter signal (V)
4
3
2
1
0
32.5
28.4
Temperature °C

Fig. 4.46. The effect of temperature changes (32.5 °C to 28.4 °C) on the constrictor response to injection of adrenaline (10^{-6}M). The upper trace shows the signal obtained from the blood flow monitor. The middle and lower traces represent arterial blood pressure and heart rate respectively. Dots on the time scale indicate one minute. On and off indicate the time that fan was turned on and off respectively.


On Off
On Off
1000 mV
T°= 32.5°C
28.4°C
32.5°C
28.4°C
40mmHg
50 beats/ min
Ad 10⁻⁶M
10⁻⁶M
10⁻⁶M
10⁻⁶M

DISCUSSION

The results of the present experiments demonstrate that within eight hours of inducing an acute inflammatory response in the rabbit knee by intra-articular injection of 4% kaolin, changes occurred in the sensitivity of α -adrenoceptors. The response to close intra-arterial injection of adrenaline demonstrates that post-junctional α -adrenoceptors become more sensitive within two hours of kaolin injection and by six hours give rise to an additional 40% reduction in blood flow compared to the response to the same dose (10^{-6}M) immediately after kaolin injection. The response to nerve stimulation might have been expected to show a similar increase in effect, but overall there was a smaller increase in the constrictor response (maximum increase ~19%). This could be explained if the inflammatory process also enhances the sensitivity of pre-junctional adrenoceptors. These are usually of the α_2 sub-type and act in a negative feed-back fashion, limiting the release of noradrenaline from sympathetic nerve endings (Vizi 1979). Preliminary experiments in previous section of this chapter suggest that such receptors are found in the rabbit knee and act as indicated above. Another possibility is that the inflammatory process enhances the mechanisms involved in the re-uptake of noradrenaline at sympathetic nerve endings and this contributes to the smaller enhancement of

the constrictor response to nerve stimulation in kaolin-injected knees.

It is noticeable that even in the saline-injected knees there occurred a gradual increase in sensitivity to injected adrenaline over eight hours (Figure 4.44). This was not due to increased sensitivity of the α adrenoceptors to cold as cooling the knee artificially with a fan and repeating the administration of adrenaline failed to show increased sensitivity in the cooled, saline-injected knee. This must therefore reflect some other factor acting to increase the sensitivity of α-adrenoceptors. One possibility is that exposure of the capsule and insertion of thermocouple probes into the synovial cavity may provoke a low grade inflammatory response over the eight hour measurement period.

The dilator response which follows nerve stimulation appeared to show a gradual rise in the kaolin-injected knee with the value at 7.5 hours having doubled compared to the control value. By contrast, in the saline- injected knee the value at 7.5 hours is only slightly increased compared to its control value. Previous work has shown that the dilator response is mediated by substance P released from C fibre afferents innervating the knee (Ferrell and Cant 1987). The increased dilator response in the inflamed knee may reflect greater release of substance P from these nerves fibres or perhaps up-regulation of substance P receptors. As substance P is known to exert pro-inflammatory actions on joint tissue (Lam and Ferrell

1989), increased release of substance P or increased sensitivity of substance P receptors may contribute to the inflammatory process.

One hypothesis which was to be tested in the present experiments was whether the vasodilation which occurs in inflamed joints could be attributed to some extent by a decrease in adrenoceptor sensitivity to noradrenaline release from sympathetic nerve endings on articular blood vessels. However, it is clear that the opposite occurs - these become sensitised by the inflammatory process. This is mitigated to some extent by the possible sensitisation of pre-junctional α_2 adrenoceptors. An additional mitigating factor may be the release of substance P from unmyelinated afferent fibres. These fibres show enhanced neural activity in acutely inflamed joints (Schaible and Schmidt 1988), and thus may contribute to vasodilation in such joints.

The present experiments have shown that acute joint inflammation induced by kaolin can influence those factors which normally regulate articular blood vessel calibre. The mechanisms responsible for altering the sensitivity of adrenoceptors (and perhaps substance P receptors) and the functional significance of this remain to be determined.

****

***

*

CHAPTER FIVE

THE INNERVATION OF THE KNEE JOINT BLOOD VESSELS IN THE CAT

SUMMARY

1. Experiments were carried out in cats anaesthetised with sodium pentobarbitone 45mg/kg. The responses of knee joint blood vessels to articular nerve stimulation and close intra-arterial injection of drugs were examined.

2. Dose/response relationships to intra-arterial injection of adrenaline, (α agonist); isoprenaline, (β agonist); acetylcholine, and histamine were obtained as well as the responses to these agonists after administration of appropriate antagonists.

3.Adrenaline and isoprenaline both induced dose dependent vasoconstriction via α-adrenoceptors in articular blood vessels. The constrictor effect of isoprenaline was reduced by phentolamine but not by propranolol.

4. Acetylcholine produced dose-dependent vasodilation in articular blood vessels. This reponse was attenuated by atropine.

5. Histamine affected knee joint blood vessels by producing an initial rise in articular blood flow followed by a fall. These effects were reduced by injection of diphenhydramine (H_1 antagonist) prior to histamine injection.

6. The posterior articular nerve (PAN) was stimulated at different intensities, and frequency response curves were obtained. Electrical stimulation of PAN produced an initial vasoconstriction (all 14 cats) during stimulation followed by a prolonged dilatation on cessation of stimulation (8 out of 14 cats). The constrictor response was increased as a function of frequency but was little altered with increasing intensity beyond a threshold level.

7. The constrictor response to electrical stimulation of PAN was markedly reduced by the α-adrenergic antagonist phentolamine(10^{-5}M), the α_1 -blocker prazosin (10^{-5}M), and guanethidine (10^{-5}M) which inhibits the release of noradrenaline, ATP, and neuropeptide Y from sympathetic nerve endings.

8. The constrictor response to PAN stimulation was unaffected by the α_2 -blocker rauwolscine and the P_2-purinoceptor desensitiser α, β, methylene ATP.

9. The dilator response was due to activation of C-fibre afferents as it could also be produced by direct electrical stimulation of L_7 dorsal roots.

10. The dilator response occurring in response to either stimulation of PAN or L_7 dorsal roots was reduced by prior intra-articular injection of 100ug of the

substance P antagonist D-Pro^{4} D-Trp^{7,9,10} -SP(4-11).

11. These results suggest that, 1) α-adrenoceptors, muscarinic receptors, and H_1 receptors are present on articular blood vessels. 2) The vasoconstrictor response to electrical stimulation of PAN is most likely to be mediated via noradrenaline acting mainly upon α_1 -adrenoceptors. 3) As the dilator response to articular nerve stimulation is reduced by a substance P antagonist, the mediator inducing this response could be substance P.

INTRODUCTION

Previous attempts at a comprehensive study of the innervation of the cat's knee joint have been by Gardner (1950) and Skoglund (1956); more limited studies have been reported by Samuel (1952). Gardner (1944) mapped the nerves to the knee joint in the cat. He described how the knee joint received its innervation and made measurements of the nerve fibre diameters of some of the nerves supplying it. Gardner's study is the only one dealing with all aspects of the anatomy of the knee joint innervation in the cat.

The knee joint is innervated by medial articular nerve (MAN), posterior articular nerve (PAN), and lateral articular nerve (LAN), (Gardner 1944, Skoglund 1956, Freeman and Wyke 1967). The PAN is the larger of the two primary articular nerves. It arises alone from the posterior tibial nerve and predominantly innervates the posterior parts of the knee joint (Freeman and Wyke 1967). The majority of fibres in PAN and MAN are unmyelinated (Langford and Schmidt 1983). It has been reported that PAN and MAN in the cat consist of about 20% myelinated and 80% unmyelinated nerve fibres. In the PAN, about half of the unmyelinated fibres are efferent sympathetic fibres and other half are afferent C-fibres (Langford 1983).

Although there are some studies concerning the innervation of the knee joint blood vessels in the cat,

little appears to be known about the action of PAN in regulating the articular blood flow.

Cobbold and Lewis (1956b) observed that electrical stimulation of the medial articular nerve (MAN) resulted in reduction of blood flow to the dog knee joint. However, the electrical stimulus parameters used by Cobbold and Lewis were not stated. In a study carried out by Ferrell and Russell (1985), it was reported that the stimulation of posterior articular nerve of the cat knee joint above C fibre threshold resulted in plasma protein extravasation into the synovial cavity.

In another study, Ferrell and Cant (1987), observed neurogenic vasodilation to PAN stimulation. They concluded that stimulation of unmyelinated articular nerve fibres produced an initial fall followed by a prolonged rise in intra-articular temperature. These findings were confirmed in a more recent study in the rabbit by Khoshbaten and Ferrell (1989). It was shown that stimulation of nerves supplying knee joint blood vessels in the rabbit resulted in an initial vasoconstriction during stimulation followed by a prolonged vasodilation on the cessation of stimulation. In other sites such as skin, it has been observed that this neurogenically mediated increase in blood vessel permeability is accompanied by dilation of these blood vessels (Couture and Cuello 1984).

As to the types of receptors present on articular blood vessels in cat and types of neurotransmitter(s) released from the nerve ending , less is known. Cobbold

and Lewis (1956b) found that close intra-arterial injection of adrenaline and noradrenaline both produced vasoconstriction while acetylcholine injection showed a considerable vasodilation. These findings were also confirmed in more recent studies on the rabbit articular blood vessels (Ferrell and Khoshbaten 1989a,b). In these studies, it was concluded that α_1 and α_2 -adrenoceptors are present within rabbit articular blood vessels, but that ß -adrenoceptors are absent. It was also found that the effect of noradrenaline appears to be mediated principally via α_1-adrenoceptors. The vasodilator effect of acetylcholine is mediated via muscarinic receptors on the endothelial layer of blood vessels.

Although it has been shown that blood vessels of the knee joint are innervated by sympathetic efferent fibres in the dog (Cobbold and Lewis 1956b), and in thecat (Ferrell and Cant 1987) whose action is to constrict these vessels, the nature of neurotransmitter(s) was not studied by these authors.

For many years, studies of the neurohumoral control of the vasculature have been dominated by the role of catecholamines released from sympathetic perivascular nerves and from the adrenal medulla into blood stream (Burnstock 1975, Burnstock et al 1980). Attention has also been paid to the cholinergic innervation of some blood vessels (Burnstock 1980). In last decade, however, nonadrenergic, noncholinergic components of the autonomic nervous system have become established. Many neurotransmitters such as ATP,

vasoactive intestinal polypeptide (VIP), substance P, dopamine and neuro-peptide Y (NPY) are now being considered for the neural control of the vasculature (Burnstock 1985).

The concept that each nerve cell makes and release only one nerve transmitter (widely known as Dale's principle) has been re-examined by Burnstock in 1976 and many others subsequently (Sneddon and Burnstock 1984, Sneddon and Westfall 1984, Burnstock and Sneddon 1985, McDonald 1988). The experimental evidence relating to the co-transmission hypothesis is outlined these papers.

Although there is abundant evidence about the neurohumoral control of many blood vessels in many vascular beds, there is still little known about the innervation of articular blood vessels.

The first evidence for involvement of noradrenaline as a neurotransmitter in articular nerves supplying the knee joint blood vessels came from the work carried out by Ferrell and Khoshbaten (1989b). It was concluded that the vasoconstrictor response to electrical stimulation of the rabbit knee joint capsule is mediated via noradrenaline acting upon α_1 -adrenoceptors.

The subsequent dilator response followed after vasoconstriction due to PAN stimulation, was abolished by intra-articular injection of the substance P antagonist (Ferrell and Cant 1987). This finding was also confirmed in a study on the rabbit (Chapter 4, section II).

In the present experiment by using laser Doppler

flowmetry, attempts were made to establish more evidence whether such vasoconstriction and neurogenic vasodilation also occur in articular blood vessels in cat, and the nature of neurotransmitter(s) released from nerve endings on these vessels.

METHODS

I. Basic procedures

1. Animals

21 adult cats were used in the present studies, their weights varying between 1.8-4.5 kg; the mean figure being 2.4 kg. These animals were starved for 12-18 hours prior to induction of anaesthesia.

2. Anaesthesia

In most cases, anaesthesia was induced by an intra-peritoneal injection of pentobarbitone ("Sagatal": May & Baker Ltd) in a dose of 45mg per kg. Maintenance of anaesthesia was by means of further intra-arterial injections of pentobarbitone in doses of 0.3-1ml (30mg/ml), depending on the weight of cat. In this way, deep anaesthesia could be maintained for long periods. An animal was judged to be deeply anaesthetised when it no longer exhibited reflex withdrawal of the forelimb on pinching the skin of that forelimb. No surgical procedures were performed until the animal had reached this level of anaesthesia.

In some experiments, induction and maintenance of anaesthesia was achieved by administration of mixture of

nitrous oxide, oxygen, and 2-4% halothane ("Fluothane": ICI) via a face mask.

3. Tracheotomy

The fur of the neck from the sternum to the jaw was shaved with clippers, and an incision made by scalpel through the skin from the hyoid bone to suprasternal notch. The skin flaps were retracted, and the thyrohyoid muscles along the midline were separated to expose the trachea. The sides of the trachea were completely cleared by blunt dissection, and a linen thread passed round it by means of an aneurysm needle. The trachea was raised by the thread and was cut halfway through between two tracheal rings craniad to the thread. The caudal edge of the cut was gripped in Spencer-Wells forceps, and a suitable glass trachea cannula was slipped into the trachea and tied in position.

4. Carotid artery cannulation

After tracheatomy, the right common carotid artery was exposed and freed from surrounding structures, particulary the vagi, and a heparinised cannula was inserted into this artery for monitoring arterial blood pressure and tied in place

The incision on the skin of the neck was then closed over with Michel clips.

5. Temperature control

During the surgical procedures and experiments, the animal core temperature was checked, and whenever needed heat was supplied by means of heating elements built into the operating table. The core temperature was monitored by a rectal thermometer. The rectal temperature was maintained at 37± 1°C. The temperature of the spinal and popliteal pools were also maintained at the same level by means of additional heating lamps.

II. Surgical procedures

When it was necessary to examine the effect of afferent C-fibres on the knee joint blood vessels, a laminectomy was necessary to expose the spinal cord, and isolation of dorsal roots L_7 and S_1. After induction of anaesthesia, tracheotomy, and carotid cannulation , the following procedures were carried out.

1. Laminectomy

The fur on the back of the animal was removed from the level of the T_{10} to the base of the tail. A midline incision was made from L_4 to the root of the tail. After then the skin flaps were retracted, the superficial lumbodorsal fascia was incised to expose the tips of the spinous processes from L_4 to S_2. The lumbar multifidus

muscle was cleared from these spinous processes by blunt dissection with a scalpel. The articular and mammillary processes of L_5 to S_1 were removed with large bone nibblers. Whilst gripping the spinous process of L_5, a small pair of bone nibblers was introduced between the L_4 and L_5, and the roof of the vertebral canal on the right side was removed from L_5 to S_1. The bone and the ligaments between the spinous processes were then removed, followed by the bone forming the roof of the vertebral canal on the left side, thus exposing the spinal cord as seen in figure 5.1. Damage to the cord was prevented by lifting the vertebral column with Spencer Wells forceps during these procedures. The lumbar multifidus muscles on both sides were removed. The spinal cord was covered with moist cotton wool, and the incision was temporarily closed with Michel clips.

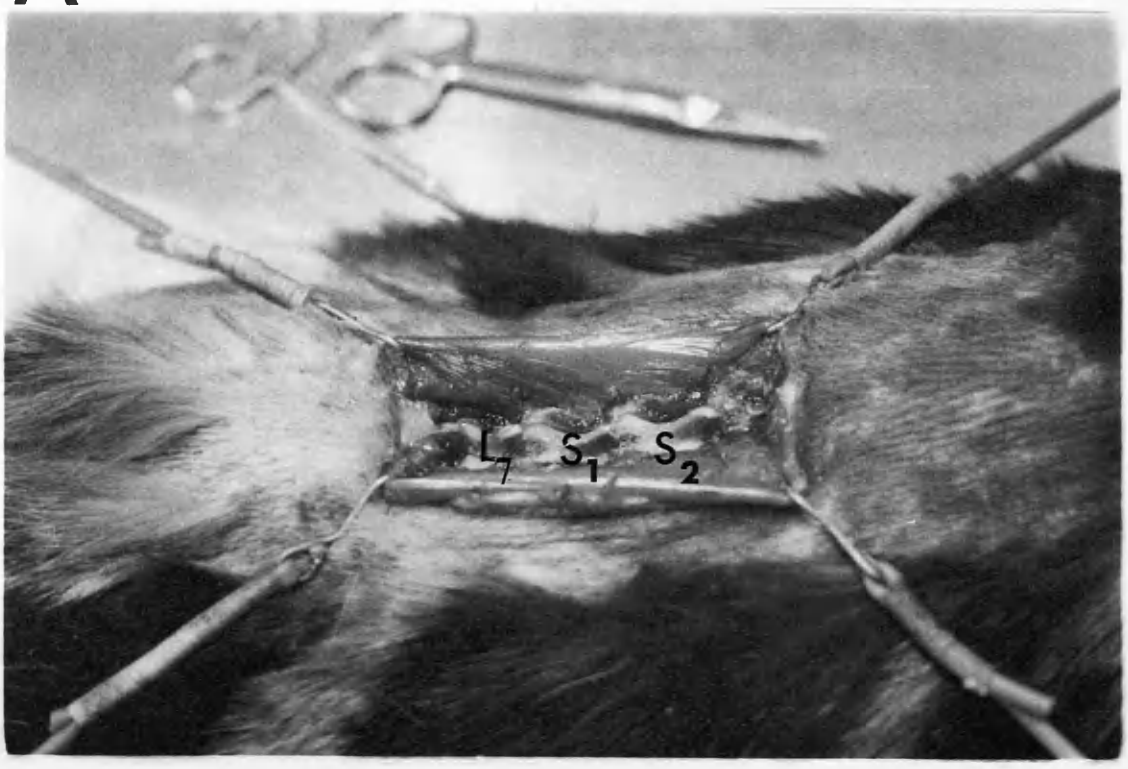
2. Posterior articular nerve (PAN) dissection

After removal of the fur from the left knee joint to the ankle, an incision was made from three centimetres craniad to the knee, down to the ankle. The skin flaps were freed by blunt dissection and retracted, exposing the gastrocnemii and the popliteal fat pad. This pad was freed from surrounding structures and removed, its blood vessels being either cauterised or cut between double ligatures. The sciatic nerve and its branches could now be seen in the popliteal fossa. The common peroneal nerve was cut as it crossed the lateral aspect of lateral gastrocnemius.

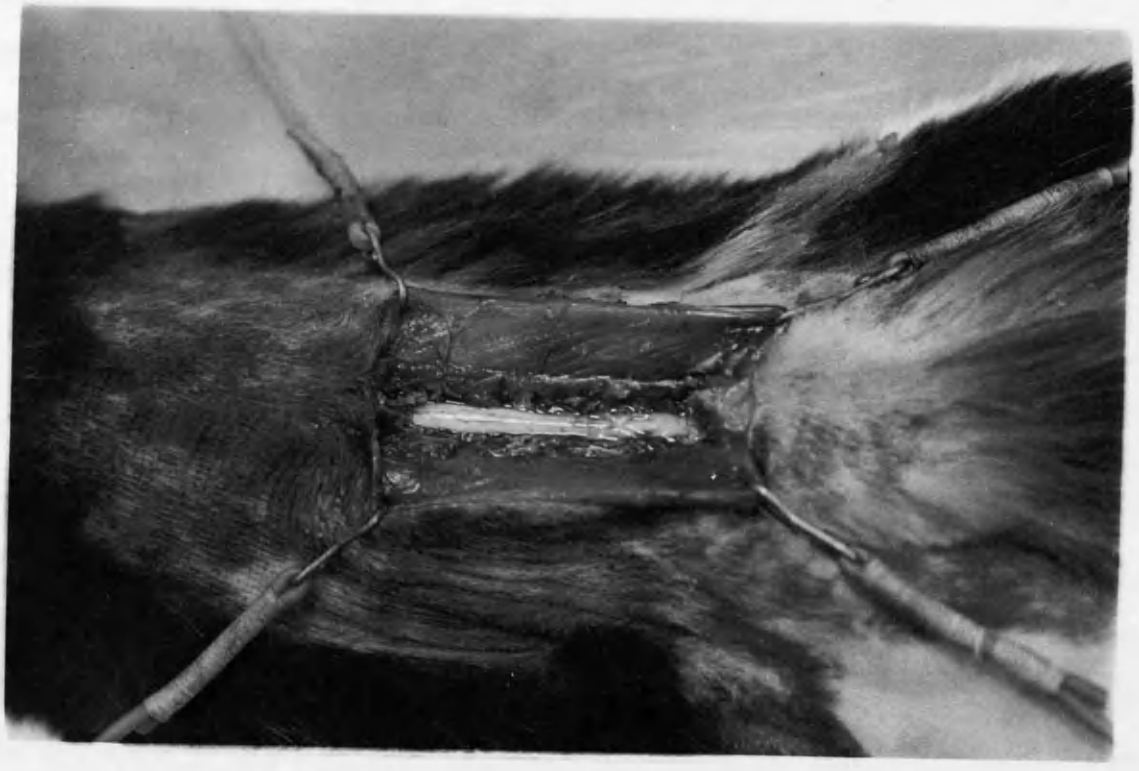
Fig. 5.1. A: First step in the laminectomy, exposure of the vertebral spinous processes. L_7 - S_2.

B: Exposure of the spinal cord after laminectomy.

A



B



The nerves to the medial and lateral gastrocnemius were then severed, and the two heads of gastrocnemius were separated from above downwards. On retraction of these, the posterior tibial nerve was visible on the deep surface lateral gastrocnemius as shown in figure 5.2 . A leash of nerves can be seen arising from the posterior tibial nerve. PAN usually forms the first branch of this leash, but in this figure it has been dissected free to simplify identification. PAN travels with the leash in the first part of its course, and then leaves it to pursue a some what tortuous course to the dorsum of the knee joint. On identification and dissection of PAN, a fine silk thread was loosely tied around it, to facilitate identification later in experiment. The posterior tibial nerve, posterior tibial leash, and nerve to soleus were all severed caudal to the point where PAN joins the posterior tibial nerve. Moist cotton wool was inserted in the popliteal fossa, and the skin incision closed with Michel clips.

3. Posterior tibial cannulation

To enable intra-arterial injections close to articular blood vessels, the posterior tibial artery was separated from surrounding structures and small branches in between the bifurcation of popliteal artery to posterior and anterior tibial arteries and 2 centimetres down were all either ligated or cauterised, then a 25G polythene cannula was inserted retrogradly, and advanced until the its tip was just distal to the branches

Fig. 5.2. Photographs of the posterior articular nerve dissection.

A: Posterior articular nerve.

B: Posterior tibial leash.

C: Posterior tibial nerve.

a
c b

supplying the knee joint.

4. Arrangement of animal in the experimental frame

On completion of the surgical procedures, the animal was located into the frame. Figure 5.3A shows an empty experimental frame, where the head holder, hind limb holder, and metal rods for holding the electrodes can be seen. The animal was rigidly mounted to the frame as demonstrated in figure 5.3B . Particular attention was given to the placing and fixation of the left hind limb.

5. Formation of the spinal paraffin pool

Once the cat had been rigidly attached to the frame, a paraffin pool was formed at the site of laminectomy (Figure 5.4). The spinal paraffin pool was formed by using a strip of X-Ray film attached to the skin flaps of the laminectomy incision by Michel clips. A circular or elleptical pool was formed when the two ends of the strip were joined up. This pool was then filled with clear liquid paraffin at a temperature of 37 °C to cover the spinal cord.

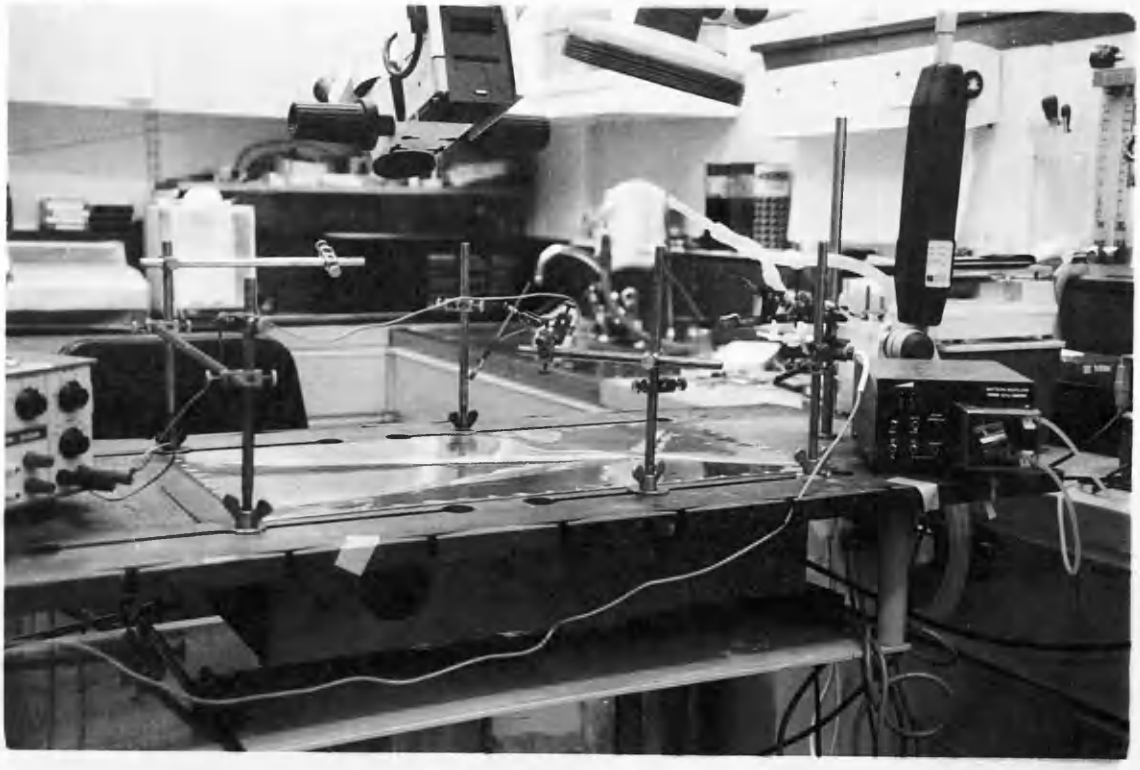
6. Retraction of the Dura and exposure of the spinal roots

With the aid of dissecting microscope, an incision was made in the dura and extended along the full length of

Fig. 5.3. A: Photograph shows an empty experimental frame.

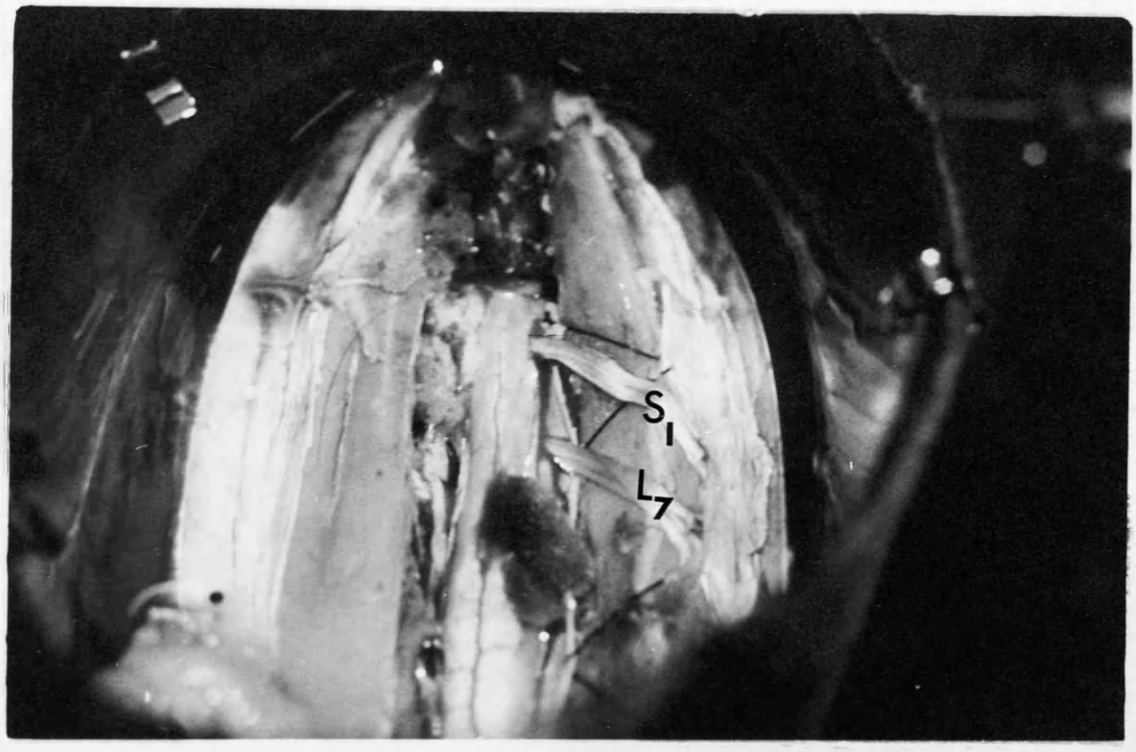
B: Photograph shows how the animal is in position for experimental procedures.

A



B

Fig. 5.4. Photographs of spinal paraffin pool. Dorsal roots L_7 and S_1 were cut close to their entry to the spinal cord and retracted.


S1
L7

the exposed spinal cord. Care was taken not to rupture or damage any of the small blood vessels overlying the spinal cord. The left and right flaps of the incised dura were then retracted and stitched onto the surrounding muscles in order to facilitate exposure of the dorsal roots. The exposed left dorsal roots L_7 and S_1 were then lifted with a glass hook and separated from each other. These dorsal roots were then sectioned close to their entry to the spinal cord (Figure 5.4).

7. Formation of the popliteal paraffin pool

This pool was simply fashioned by rigidly positioning the hind limb at a knee joint angle of about 120°, and retracting the popliteal skin flaps and attaching these to the frame. Warmed liquid paraffin (37°C) was then poured into the popliteal fossa(Figure 5.5).

8. Locating the laser probe

The fibre optic probe (2mm in diameter), was either located on the external surface of posterior capsule (Figure 5.5) or a smaller probe (0.9mm in diameter) was inserted (via a 18G needle) from the antero-lateral side of knee into the joint cavity and advanced dorsally to contact the internal surface of the posterior capsule (Figure 5.6).

9. Stimulation of L_7 and S_1.

Fig. 5.5. Photograph of popliteal paraffin pool. Laser probe is shown placed on theexternal surface of posterior joint capsule.

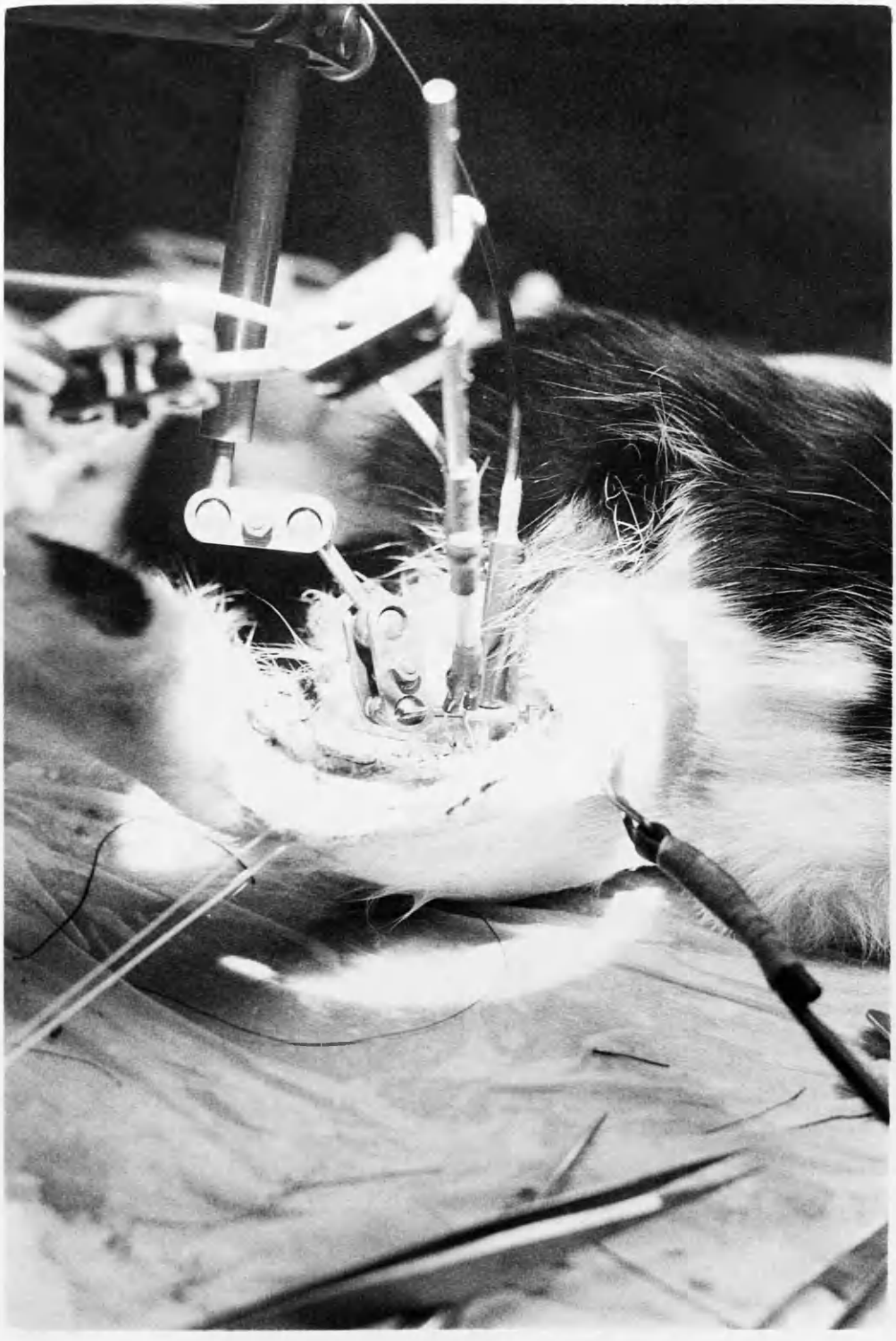
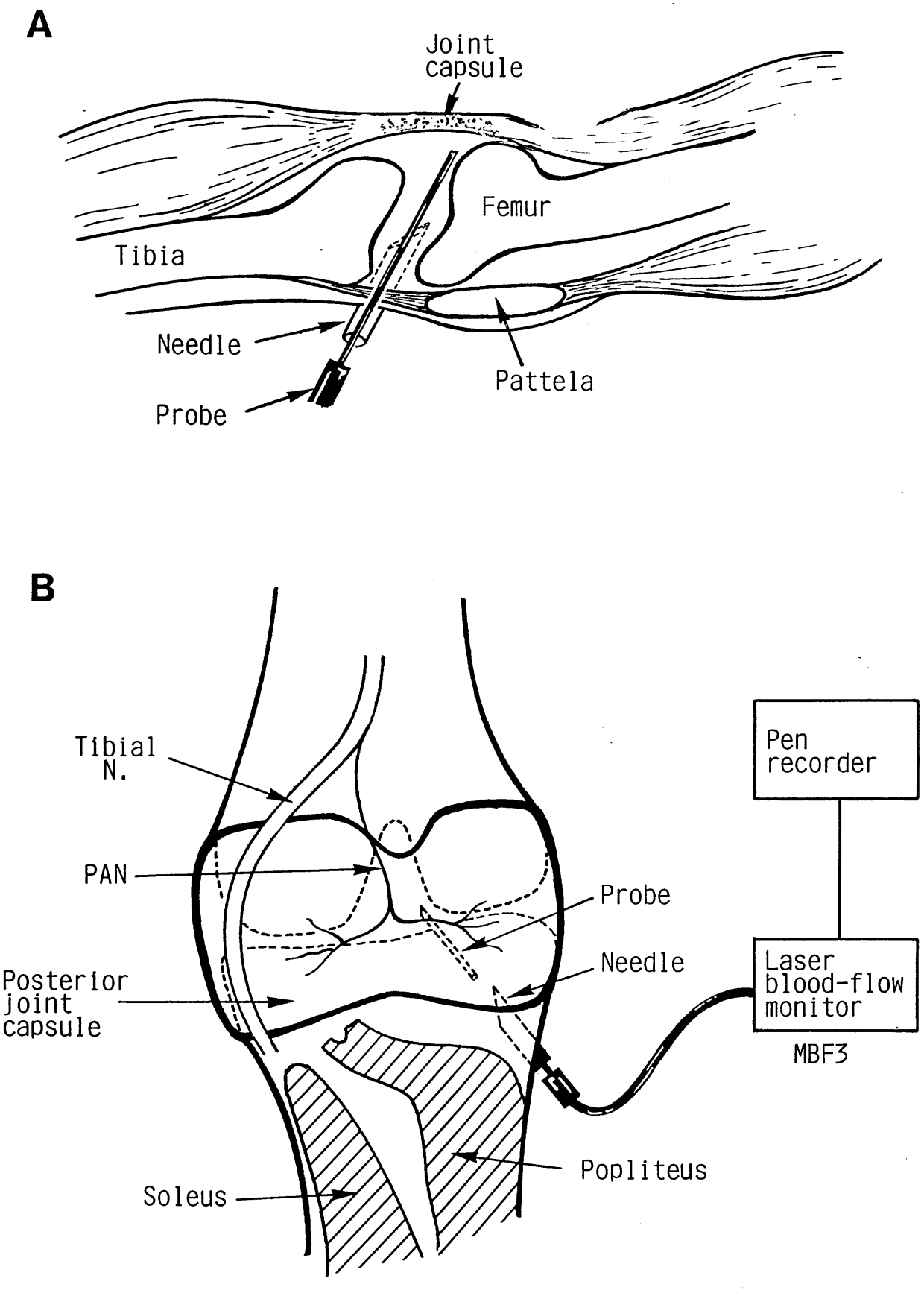


Fig. 5.6. Diagram of the experimental arrangement to show where the laser probe is located, A; view of lateral aspect of cat knee joint, B; posterior aspect of knee joint in cat. Laser probe was inserted from antero-lateral side through the infra-patellar region.


A
Joint capsule
Femur
Tibia
Needle
Pattela
Probe
B
Tibial N.
PAN
Posterior joint capsule
Probe
Needle
Soleus
Popliteus
Pen recorder
Laser blood-flow monitor
MBF3

Before cutting the PAN, L_7 and S_1 dorsal roots were located on a pair of silver electrodes with an inter-electrode distance of two and half millimetres. Electrodes were connected to a Harvard advanced stimulator which produced square wave pulses whose duration, intensity, and frequency could be varied.

10. Stimulation of PAN

To see the direct effect of PAN stimulation, after the formation of the popliteal paraffin pool, under the dissecting microscope the PAN was located on a pair of silver electrodes connected to the stimulator and then was cut while the knee joint blood flow, arterial blood pressure, and heart rate were monitoring on th pen recorder.

11. Termination of the experiment

At the end of the experiment the animal was painlessly killed by an intra-arterial overdose injection of sodium pentobarbitone, 3-5ml, 200mg/ml (Euthatal, May & Baker Ltd.).

12. Statistics

Statistical data analysis was carried out by either paired or unpaired t test. An F test was also used to test

the assumption of homogeneity of variances. Where this exceeded tabled F values, modified t values were generated using the formula described by Phillips (1978). All data expressed on graphs are means $\pm$ S.E.M. Differences between means were considered significant if the P values were 5% or less.

RESULTS

1. Drug injection

a. The effect of adrenaline on articular blood vessels

Intra-arterial injection of adrenaline resulted in a dose-dependent vasoconstriction of the knee joint blood vessels (Figure 5.7). Although the response at each dose did not significantly differ from the response evoked by the preceding dose except for the 10^{-8}M dose, they all differed significantly from control.

b. The effect of isoprenaline (ISO)

The presence of ß receptors was tested by intra-arterial injection of isoprenaline, a ß-agonist. A constrictor response was observed following the injection (Figures 5.8, 5.10A). The injection of ISO was also investigated after injections of both an α-antagonist, phentolamine and a ß-antagonist, propanolol (Figures 5.9, 5.10B). The constrictor effect of ISO illustrated in figure 5.10A was mediated via α-adrenoceptors as it could be reduced by phentolamine (10^{-5}M) while propanolol (10^{-4}M) did not significantly change the response although the fall in blood pressure due to ISO injection was

Fig. 5.7. Intra-arterial injection of adrenaline (10^{-9}M-10^{-4}M) produces dose/dependent vasoconstriction of articular blood vessels. n= 6-8, * $P<0.05$.

The concentrations can be converted to the mass of drug injected by multiplication of that concentration by $2x10^{-4}$ in this and subsequent figures.

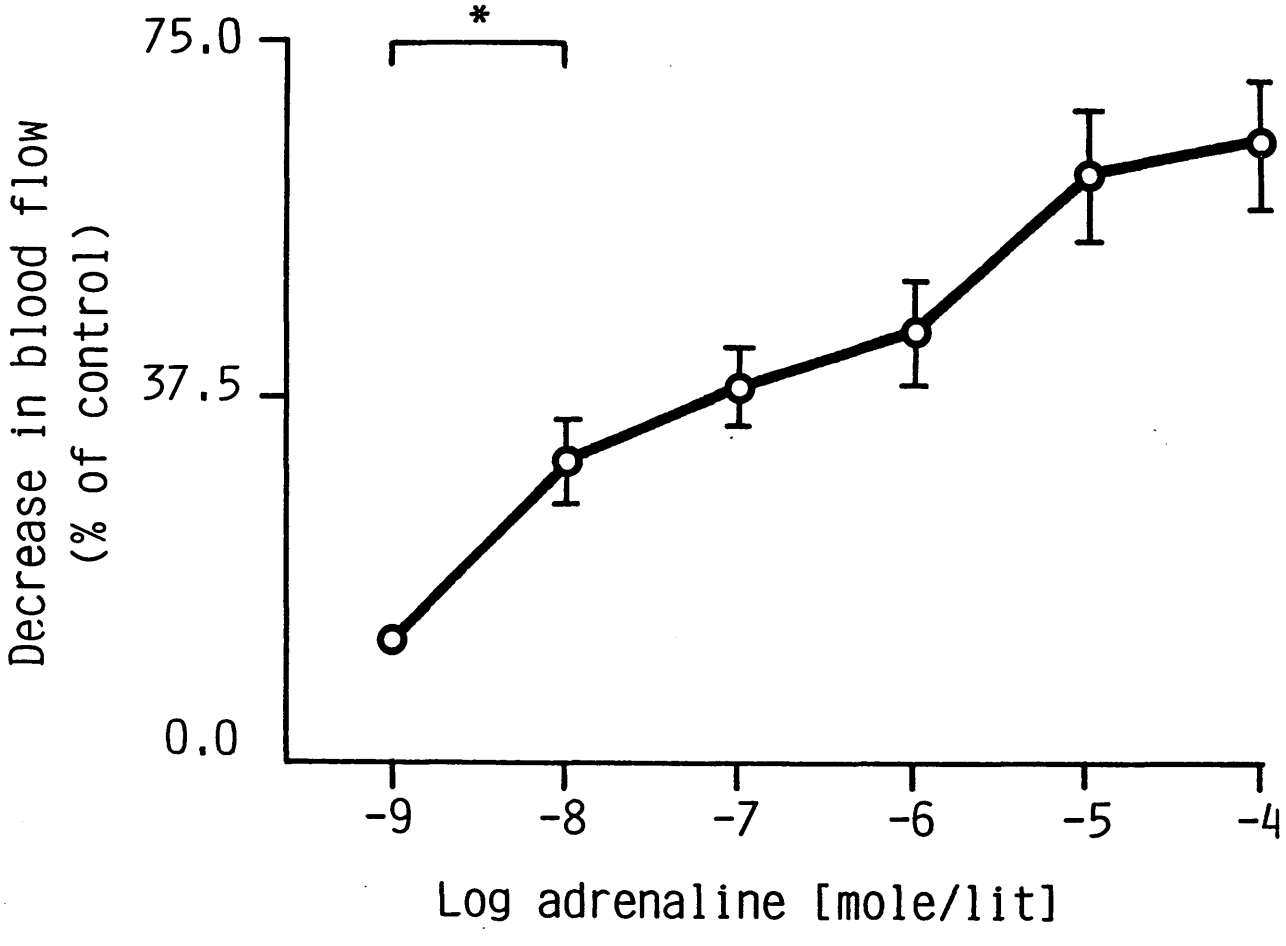

*
75.0
37.5
0.0
Decrease in blood flow
(% of control)
-9
-8
-7
-6
-5
-4
Log adrenaline [mole/lit]

Fig. 5.8. The effect of close intra-arterial injection of isoprenaline (10^{-8}M - 10^{-4}M) on articular blood vessels (panel A), systolic blood pressure (panel B), and diastolic blood pressure (panel C). n=5, * $P<0.05$, ** $P<0.01$.

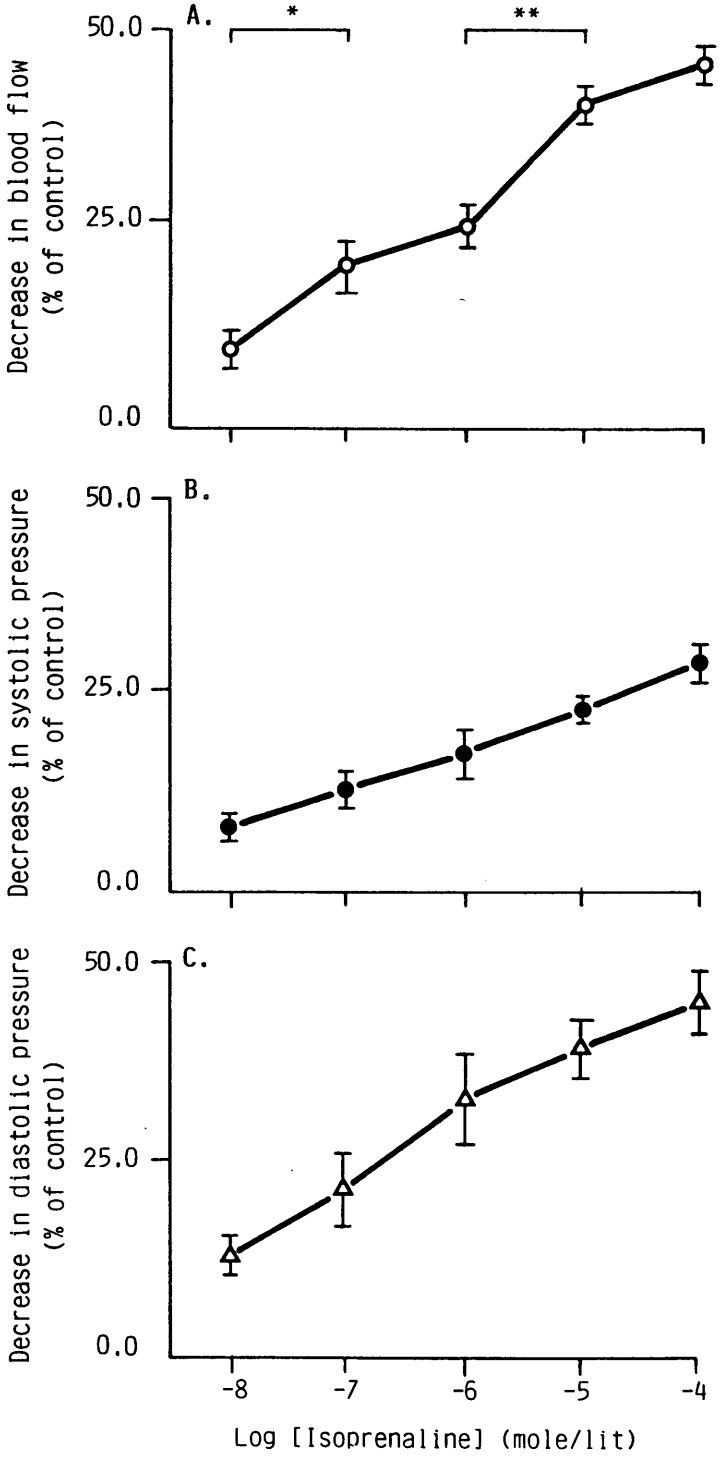
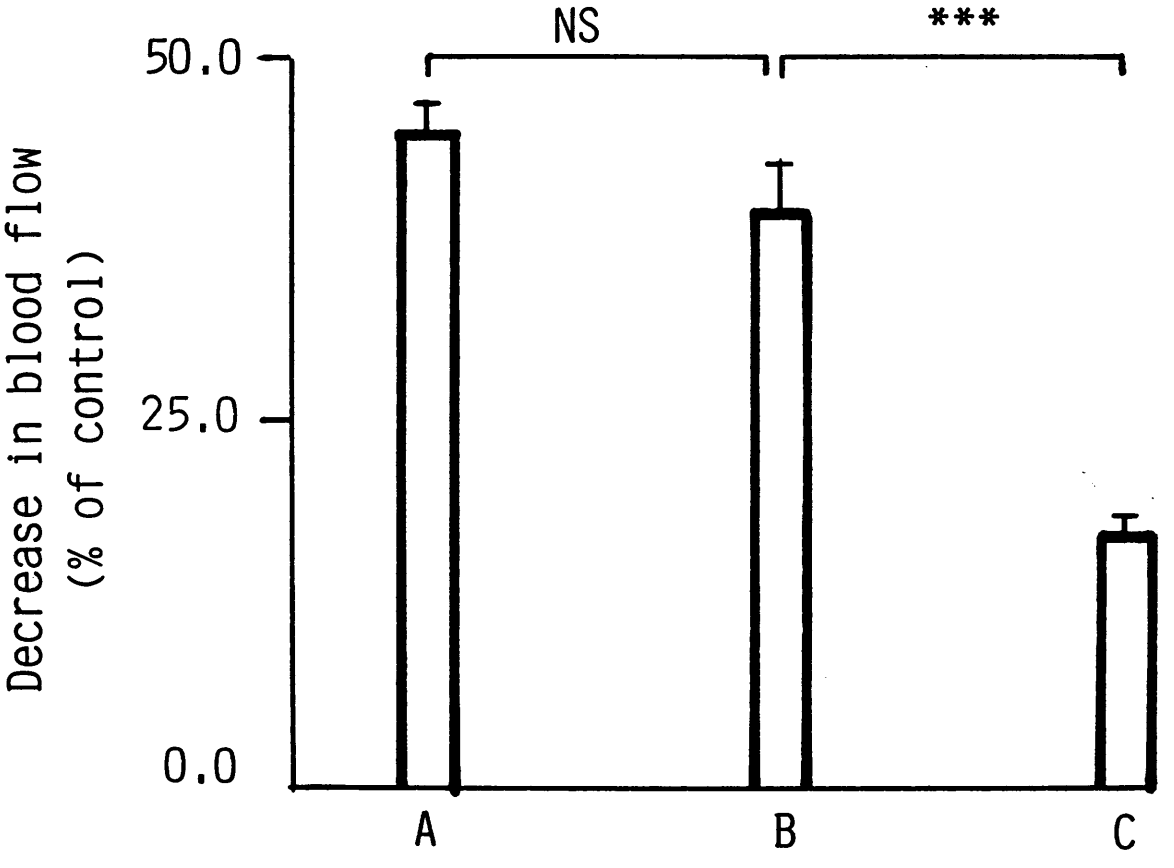

A.
*
**
50.0
25.0
0.0
Decrease in blood flow
(% of control)
B.
50.0
25.0
0.0
Decrease in systolic pressure
(% of control)
C.
50.0
25.0
0.0
Decrease in diastolic pressure
(% of control)
-8
-7
-6
-5
-4
Log [Isoprenaline] (mole/lit)

Fig. 5.9. Responses of articular blood vessels were compared between close intra-arterial injection of isoprenaline (10^{-4}M),before (A) and after injection of propranolol,10^{-4}M (B), and phentolamine, 10^{-5}M (C). There is no significant response between injection of isoprenaline before and after propranolol. But the response was obviously reduced by phentolamine injection prior to isoprenaline. *** $P<0.001$, n= 5 - 7.


NS
***
50.0
25.0
0.0
Decrease in blood flow
(% of control)
A
B
C

attenuated(Figure 5.10).

c. The effect of acetylcholine (ACh)

The presence of cholinergic receptors was investigated by intra-arterial injection of ACh. As illustrated in figure 5.11, there was a rise in articular blood flow following the injection of ACh. The dilator effect of ACh illustrated in figure 5.12 shows a dose-dependent vasodilation of these vessels. However there was less response at a dose of 10^{-6}M compared to the 10^{-7}M dose. Monitoring arterial systolic and diastolic pressures during the experiments, permitted assessment of whether there is any systemic effect, following ACh injection. Obviously, by injecting 0.2ml ACh even at a dose 10^{-8}M, arterial diastolic pressure was reduced. This could be because of a temporary systemic vasodilation since the heart rate did not change significantly (Figures 5.11, 5.12). Injection of atropine (muscarinic blocker) prior to ACh injection, attenuated the vasodilator effect of ACh (Figure 5.13). So the dilator response of ACh was mediated via muscarinic receptors.

d. The effect of histamine

Although histamine is widely known as a vasodilator agent in blood vessels (Haddy 1960, Powell and Brody 1976), there are reports describing the use of histamine to raise the tone of blood vessels(Kennedy and Burnstock

Fig. 5.10. Traces show the effect of isoprenaline on the knee joint blood vessels and arterial blood pressure before (panel A) and after (panel B) propranolol(10^{-4}M).

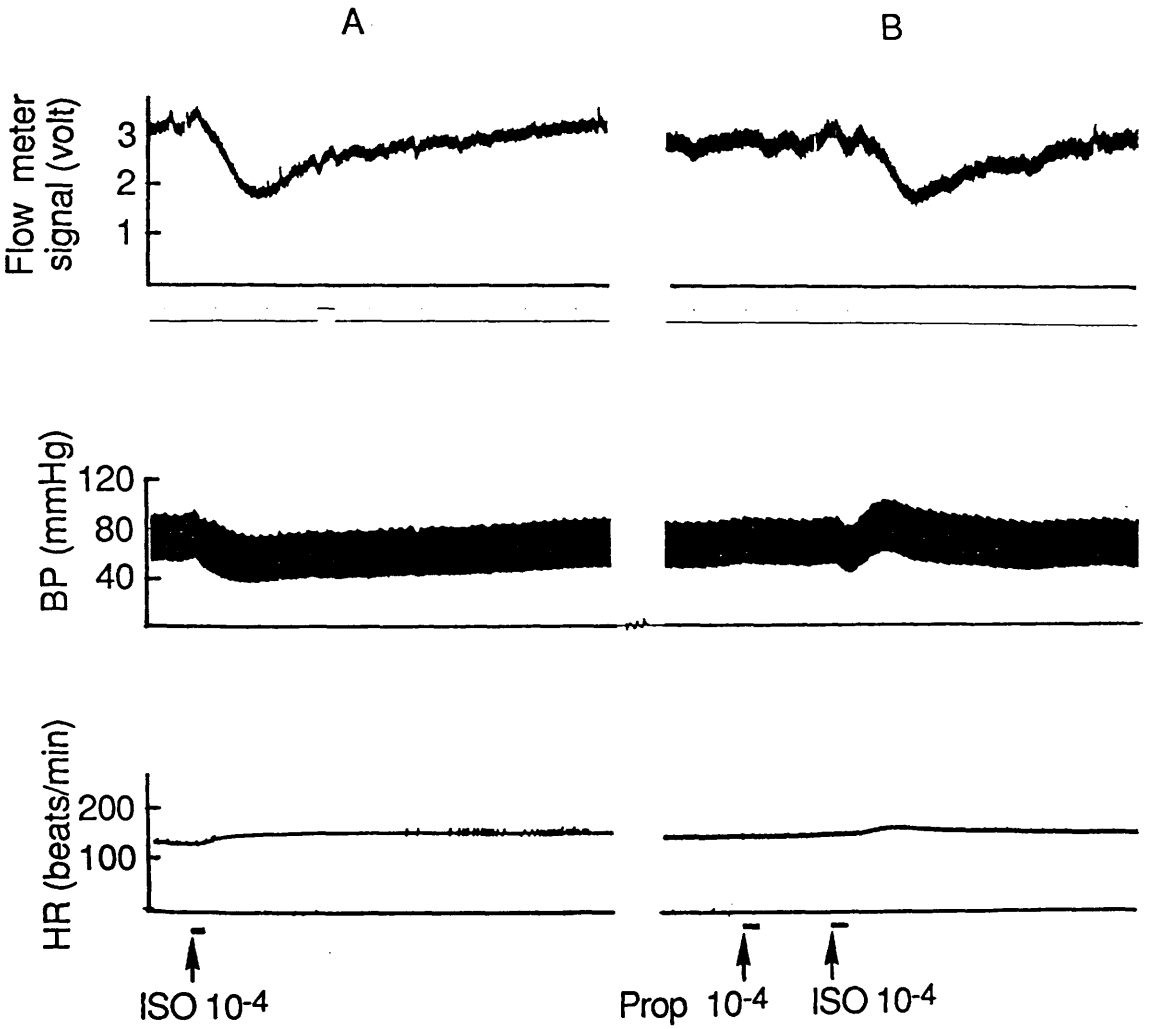

A
B
Flow meter signal (volt)
3
2
1
BP (mmHg)
120
80
40
HR (beats/min)
200
100
ISO 10-4
Prop 10-4
ISO 10-4

Fig. 5.11. Trace shows that intra-arterial injection of ACh (10^{-8}M) produce vasodilation in articular blood vessels (top trace). Arterial blood pressure transiently falls down but there is no change in heart rate. Electrical stimulation of the L_7 dorsal roots also induces dilatation of these blood vessels.

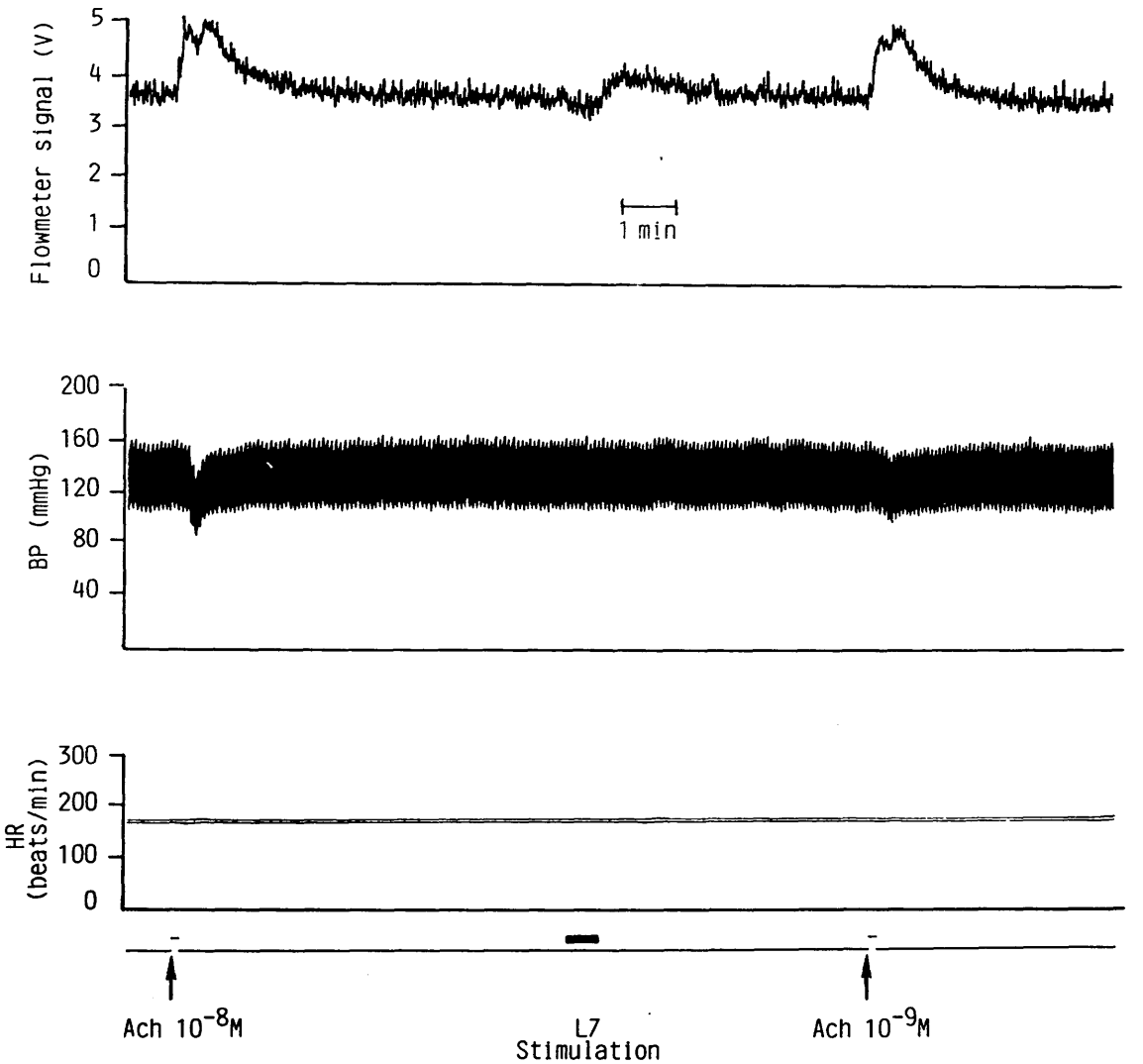

Flowmeter signal (V)
5
4
3
2
1
0
1 min
BP (mmHg)
200
160
120
80
40
HR (beats/min)
300
200
100
0
Ach 10^{-8}M
L7 Stimulation
Ach 10^{-9}M

Fig. 5.12. A: the effect of intra-arterial administration of different doses of ACh on articular blood vessels. ACh produces a vasodilator response in these blood vessels.

B: The effect of ACh injection on arterial diastolic pressure. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, n=5-7.

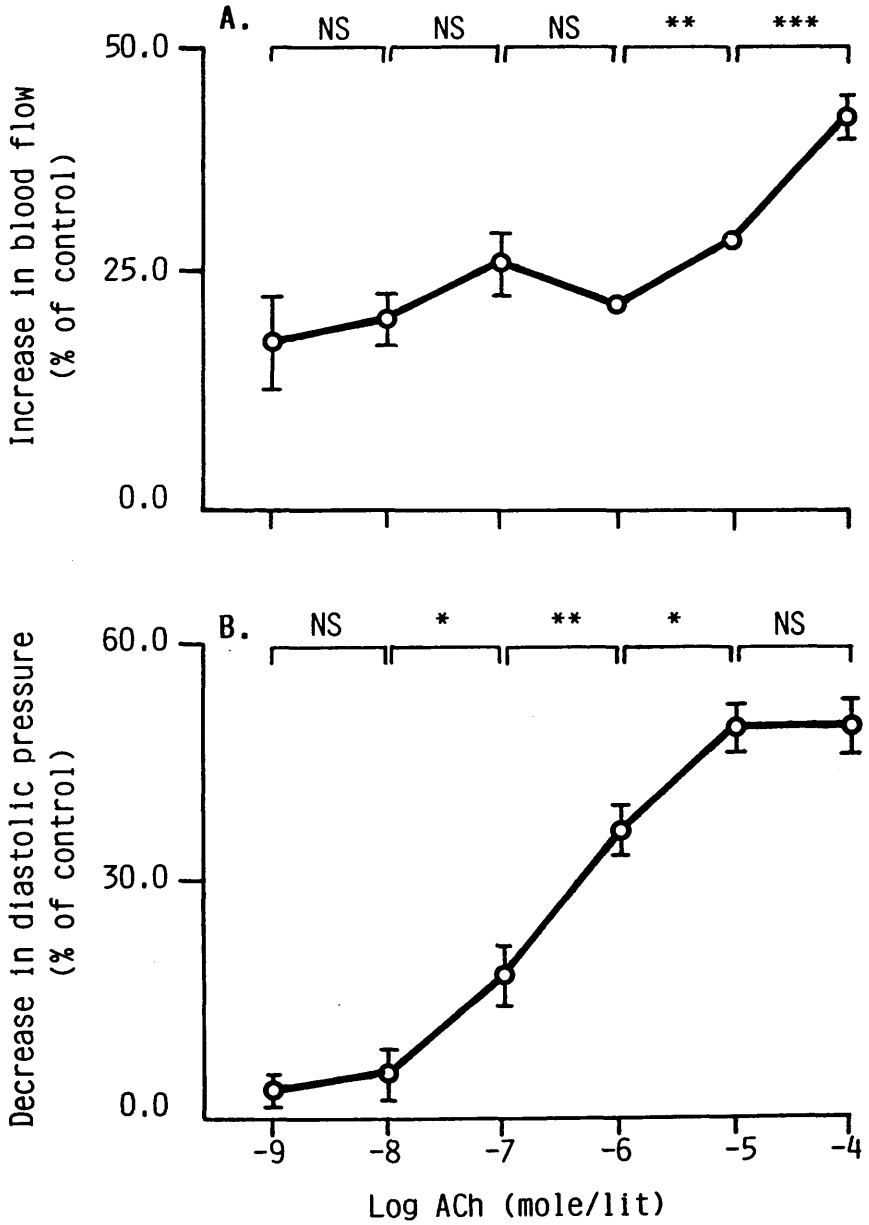
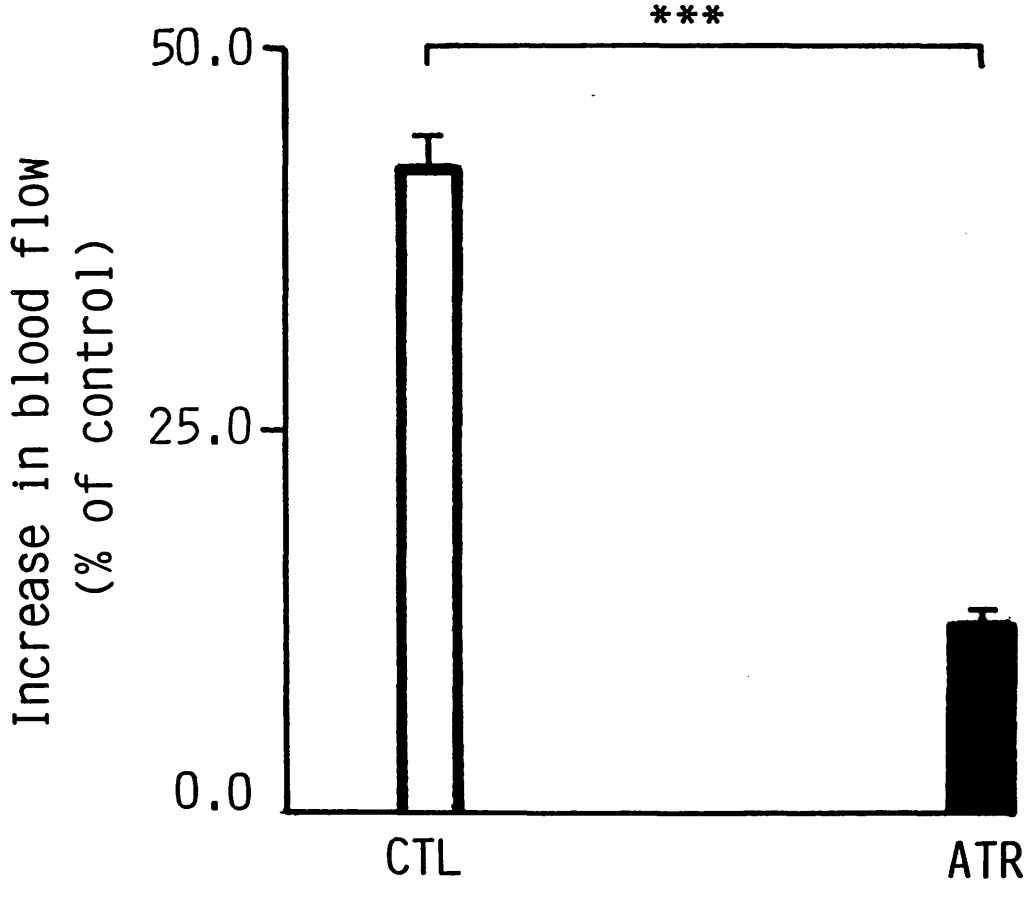

A.
NS
NS
NS
**
***
50.0
25.0
0.0
Increase in blood flow
(% of control)
B.
NS
*
**
*
NS
60.0
30.0
0.0
Decrease in diastolic pressure
(% of control)
-9
-8
-7
-6
-5
-4
Log ACh (mole/lit)

Fig. 5.13. Histograms compare the effect of ACh 10^{-4}M on articular blood vessels before (CTL) and after intra-arterial injection of atropine 10^{-4}M (ATR). This effect was significantly reduced. n= 5, *** P<0.001.


***
50.0
25.0
0.0
Increase in blood flow
(% of control)
CTL
ATR

1985). In the present study, intra-arterial injection of histamine resulted in a biphasic response, an initial rise followed by a prolonged fall in blood flow in articular blood vessels (Figure 5.14B). As illustrated in figure 5.14A the dilator response was most obvious at low doses (10^{-11}M to 10^{-8}M) while the constrictor effect became significant from 10^{-8}M, and this response was dose-dependent where as the dilator response was not (Figure 5.15). Following injection of histamine 10^{-8}M and up, there were falls in arterial systolic and diastolic pressures and rises in heart rate. These change were the systemic effects of histamine which entered the circulation. As illustrated in figure 5.14B, whilst the systemic effects of histamine started to reverse, the constrictor response to histamine was still increasing. Thus the possibility that the fall in articular blood flow was only due to decrease in systemic blood pressure appears unlikely. Injection of diphenhydramine (H_1-blocker) reduced both effects of histamine on articular blood vessels and systemic blood pressure (Figure 5.16).

2. Nerve stimulation

a. Denervation of the posterior articular nerve (PAN)

After dissecting the PAN free from surrounding tissue, it was placed over double silver chloride hook electrodes in the popliteal paraffin pool. Thereafter, it

Fig. 5.14. Panel A: shows signal representing the vasodilator response to injection of histamine at low doses (10^{-9}M).

Panel B: shows the biphasic effect of histamine in higher doses (10^{-5}M).

Panel C: shows the reduction of response to histamine in present of diphenhydramine. Middle and lower traces show arterial blood pressure and heart rate changes respectively.

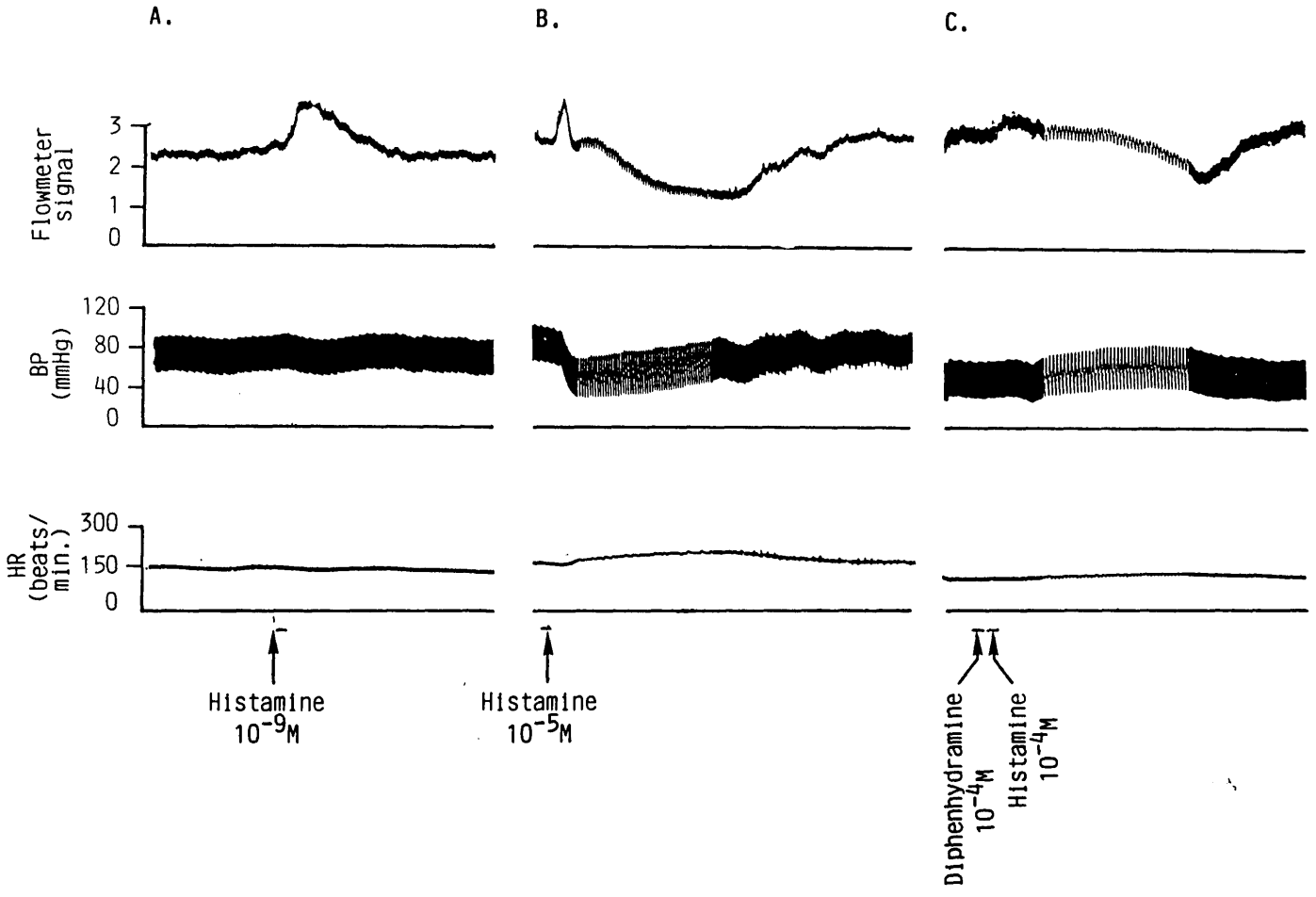

A.
B.
C.
Flowmeter signal
3
2
1
0
BP (mmHg)
120
80
40
0
HR (beats/ min.)
300
150
0
Histamine 10^{-9}M
Histamine 10^{-5}M
Diphenhydramine 10^{-4}M
Histamine 10^{-4}M

Fig. 5.15. A: Intra-arterial injection of histamine produces dilator response in articular blood vessels.

B: Following the dilatory response to histamine injection there is also vasoconstriction in the same blood vessels.

C: Injection of histamine causes a fall in arterial diastolic blood pressure at the same time. * $P<0.05$, ** $P<0.01$. n= 5 - 8.

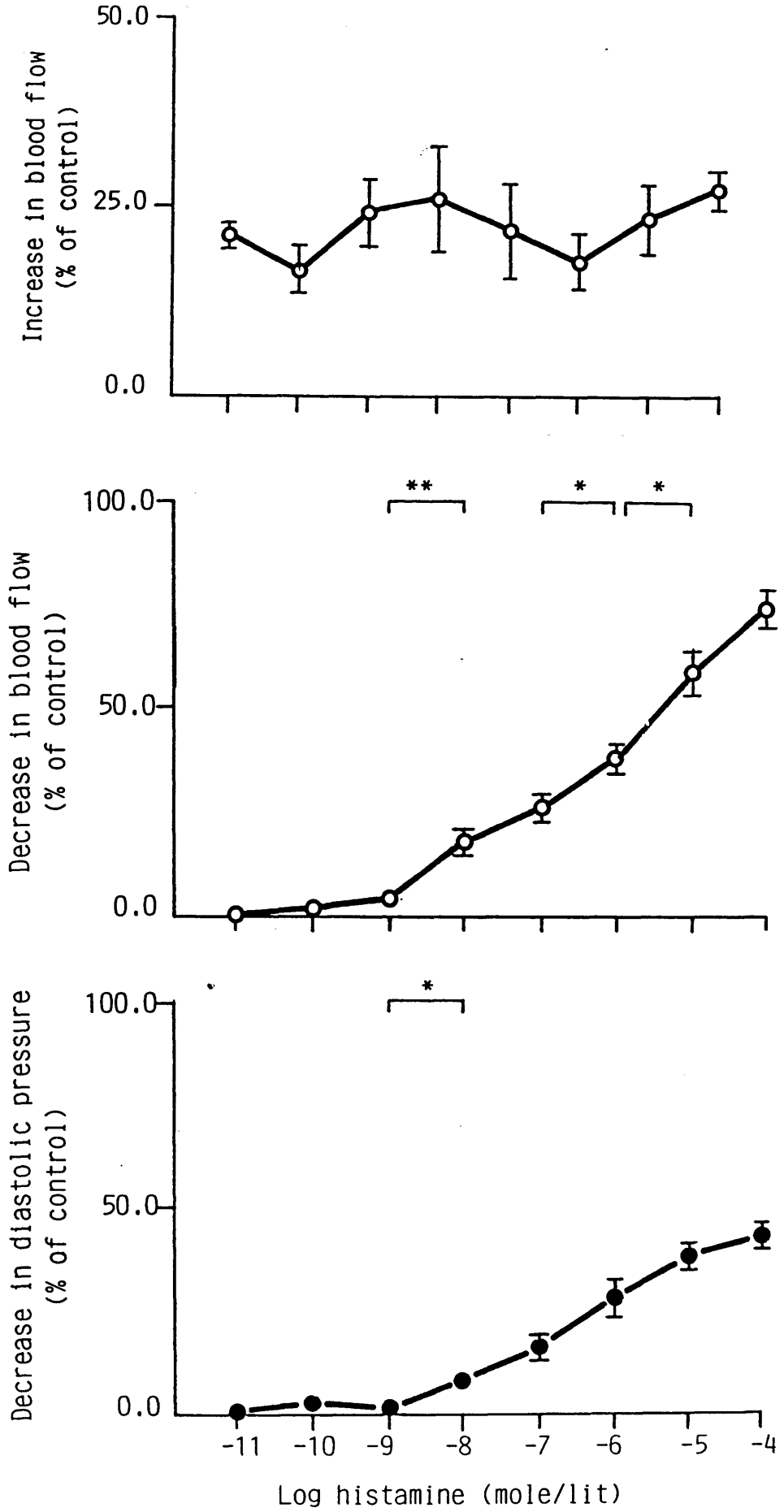

50.0
25.0
0.0
Increase in blood flow
(% of control)
**
*
*
100.0
50.0
0.0
Decrease in blood flow
(% of control)
*
100.0
50.0
0.0
Decrease in diastolic pressure
(% of control)
-11
-10
-9
-8
-7
-6
-5
-4
Log histamine (mole/lit)

Fig. 5.16. Responses to close intra-arterial injection of histamine (10^{-4}M) before (filled histogram) and after (open histogram) injection of diphenhydramine (10^{-4}). Constrictor response (A) followed by the dilator response (B) of histamine was significantly affected by diphenhydramine. The percentage fall in diastolic pressure in result of histamine injection was also attenuated(C). n= 5, * $P<0.05$, ** $P<0.01$.

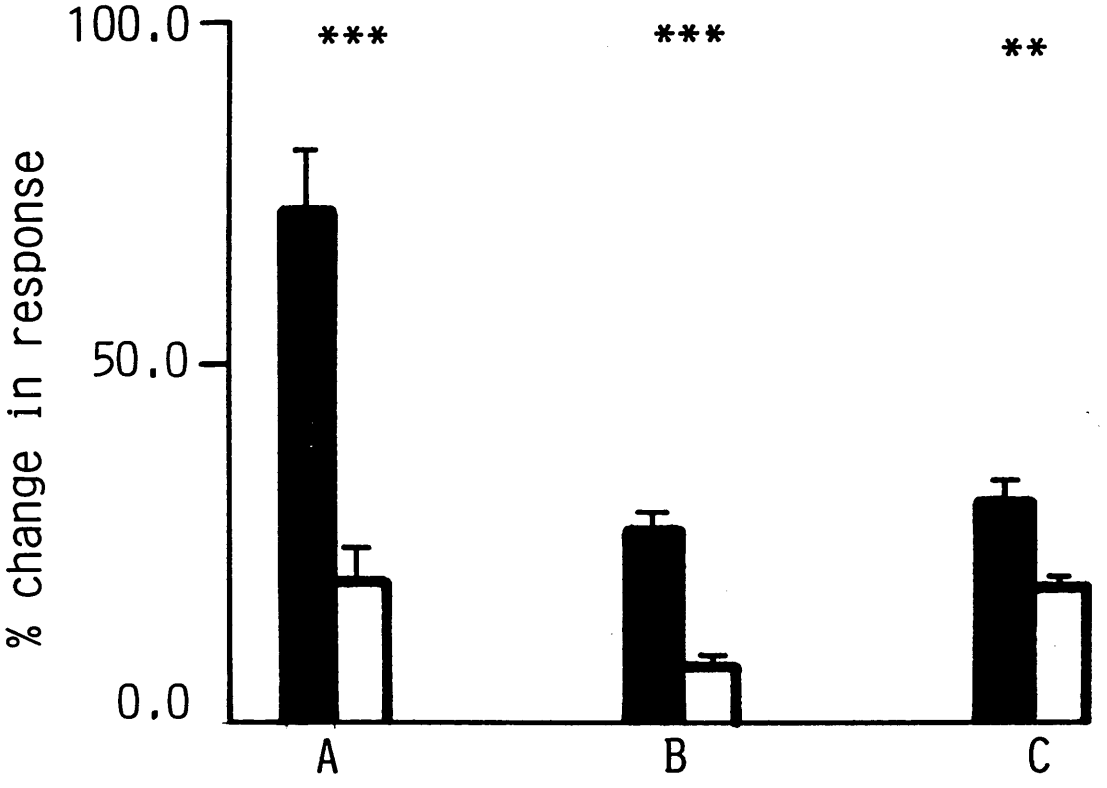
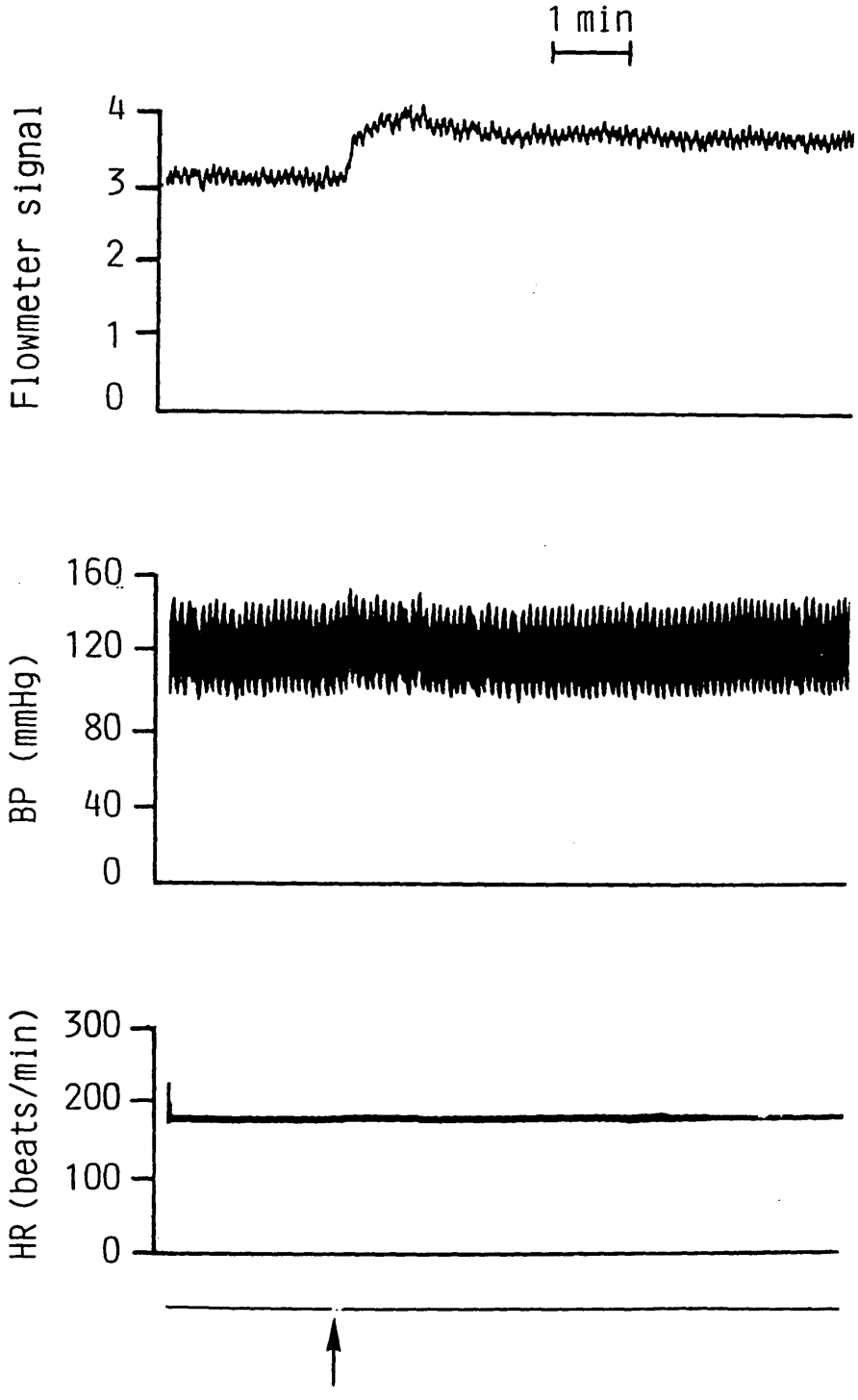

100.0
50.0
0.0
% change in response
***
***
**
A
B
C

Fig. 5.17. Acute transection of PAN induces a rise in knee joint blood flow. This could be the result of removal of sympathetic basal tone from these vessels. Arrow shows the point of transection.


1 min
Flowmeter signal
4
3
2
1
0
BP (mmHg)
160
120
80
40
0
HR (beats/min)
300
200
100
0

was cut proximally. As shown in figure 5.17, in some experiments transection of the PAN resulted in an obvious rise in blood flow to the joint.

b. Stimulation of PAN

In 8 out of 14 cats, stimulation of articular nerve fibres resulted in a characteristic pattern of initial fall in the blood flow of the knee joint during stimulation followed by a prolonged vasodilation on cessation of stimulation (Figure 5.18). In all 14 cats the constrictor response was observed on electrical stimulation of PAN (table 8).

In order to check whether the responses to PAN stimulation only reflected changes occurring in articular blood vessels, in experiments using the intra-articular probe, a film of black polythene was placed between the outer surface of posterior capsule and the muscles overlying the posterior capsule (Figure 5.19). In other experiments with the laser probe on the external surface of posterior capsule, the polythene film was located between the capsule and condyle of femur via a small incision through the capsule (Figure 5.20). When electrical stimulation was applied before and after removal of the polythene film, no change was observed with either of these manoeuvers.

Responses to PAN stimulation were obtained both by increasing the number of pulses per second and also by increasing the stimulus voltage(Figure 5.21). These showed

Fig. 5.18. The top trace shows the signal obtained from the blood flow monitor in response to electrical stimulation of the PAN. Vasoconstriction occurs during stimulation followed by dilation on cessation of stimulation. As the PAN was sectioned proximally heart rate and arterial blood pressure remained constant. Small bars represent the time of stimulation (10V, 10HZ, 1msec).

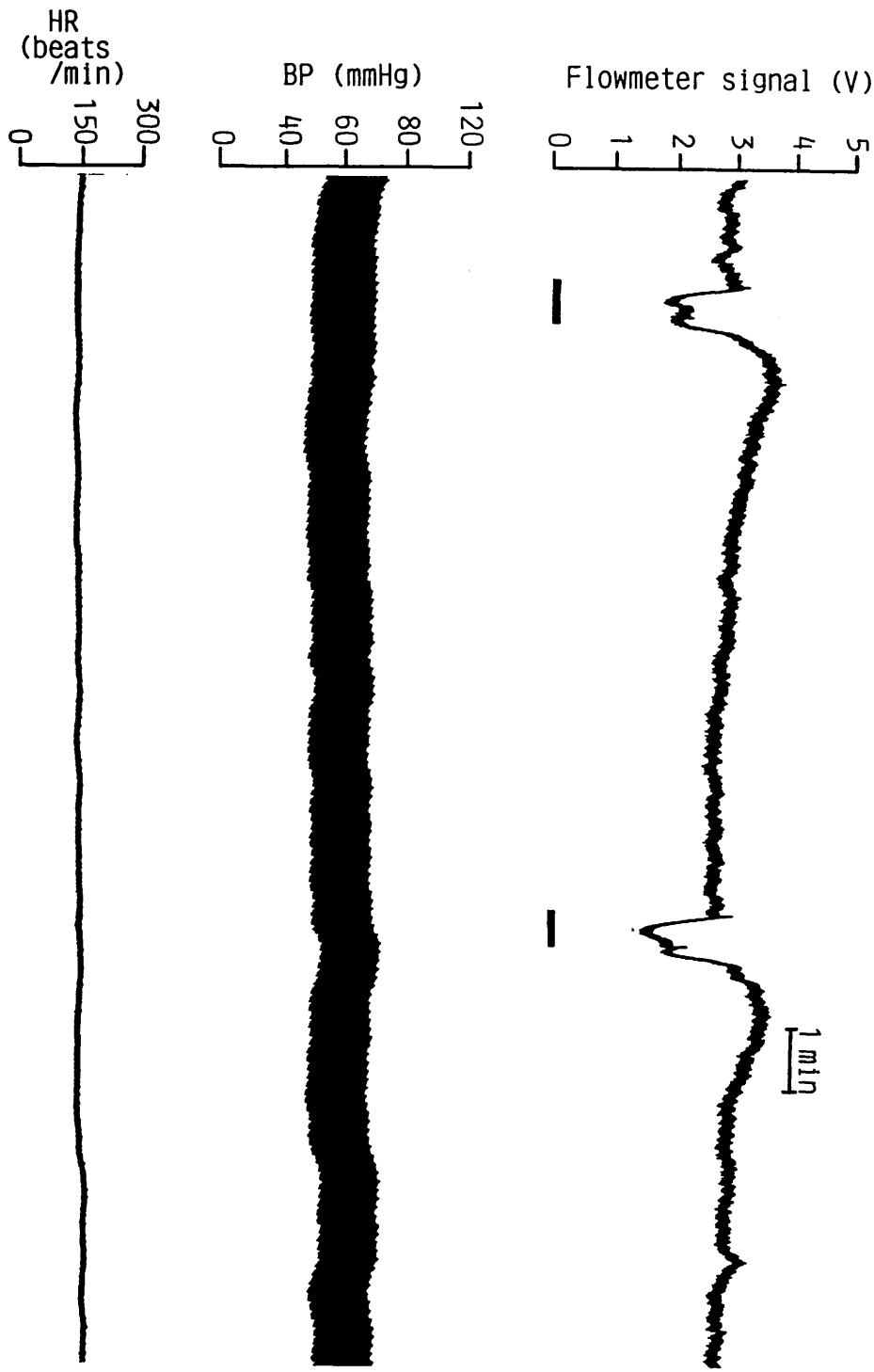

HR
(beats
/min)
0
150
300
BP (mmHg)
0
40
60
80
120
Flowmeter signal (V)
0
1
2
3
4
5
1 min

Fig. 5.19. Responses to stimulation of PAN (10V, 10HZ, 1msec) before and after locating of black polyethylene film between outer surface of posterior capsule and covering tissues e.g.muscles and skin. Flow probe was inserted antro-laterally and advanced until it contacted the internal surface of posterior capsule. Arrow shows the time that black polyethylene film was put in place. S bars represent the time of stimulation.

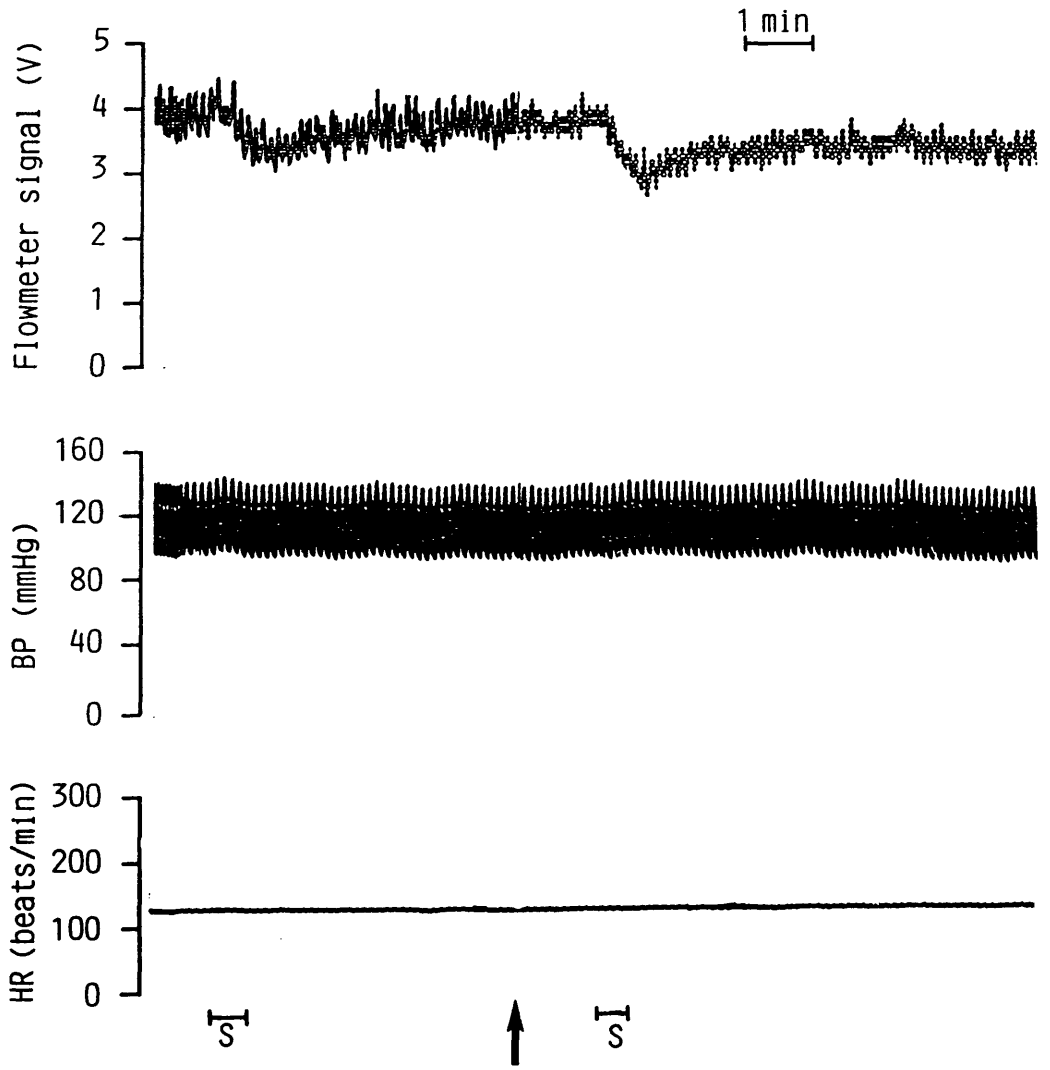

1 min
Flowmeter signal (V)
5
4
3
2
1
0
BP (mmHg)
160
120
80
40
0
HR (beats/min)
300
200
100
0
S
S

Fig. 5.20. Responses to stimulation of PAN (10V, 10HZ, 1msec) before and after removal of black polyethylene film which was located between internal surface of posterior capsule and femoral condyle. The probe was placed on the external surface of posterior capsule. Arrow shows the time that the black polythene was removed. Bars indicate the time of stimulation.

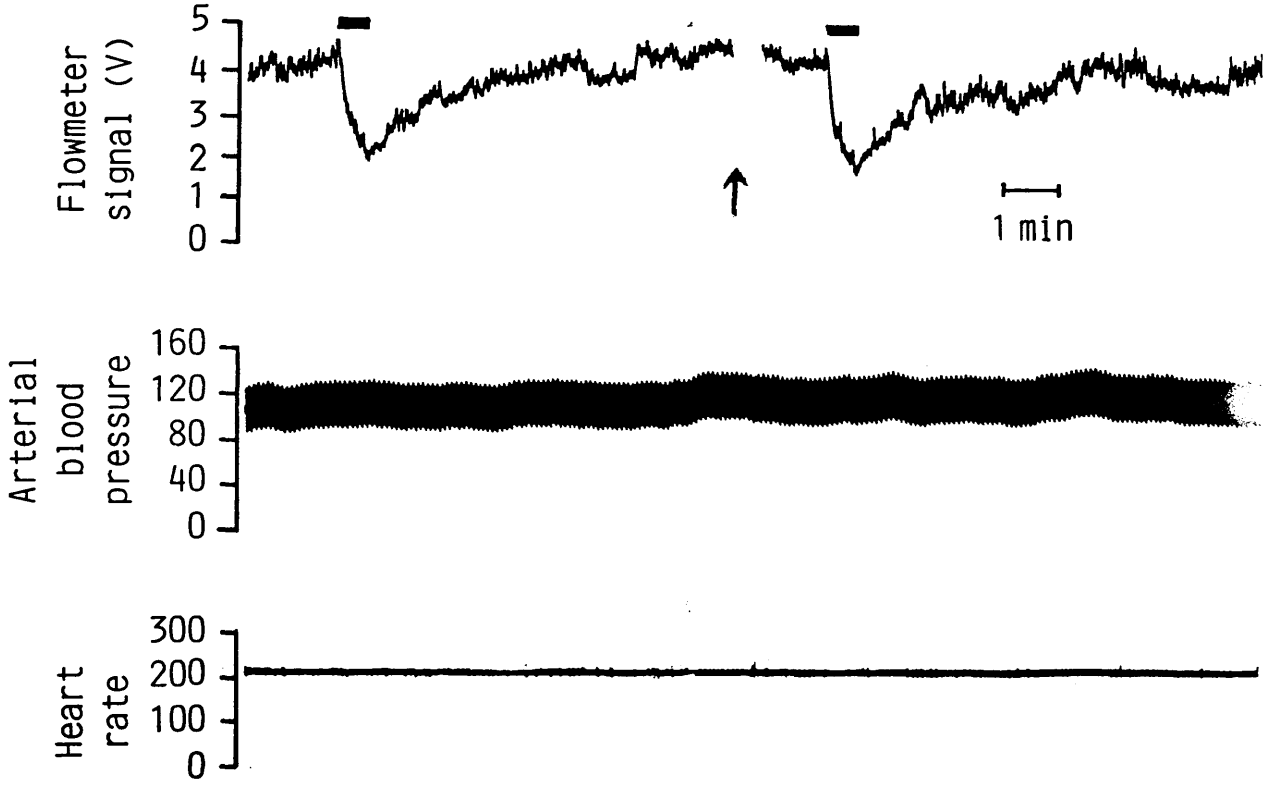

Flowmeter signal (V)
5
4
3
2
1
0
1 min
Arterial blood pressure
160
120
80
40
0
Heart rate
300
200
100
0

Fig. 5.21. This graph plots the constrictor response against frequency of PAN stimulation with 5Volt (●), and 10Volt (O) magnitudes. No significant changes are observed between two different voltages. n= 5 - 12.

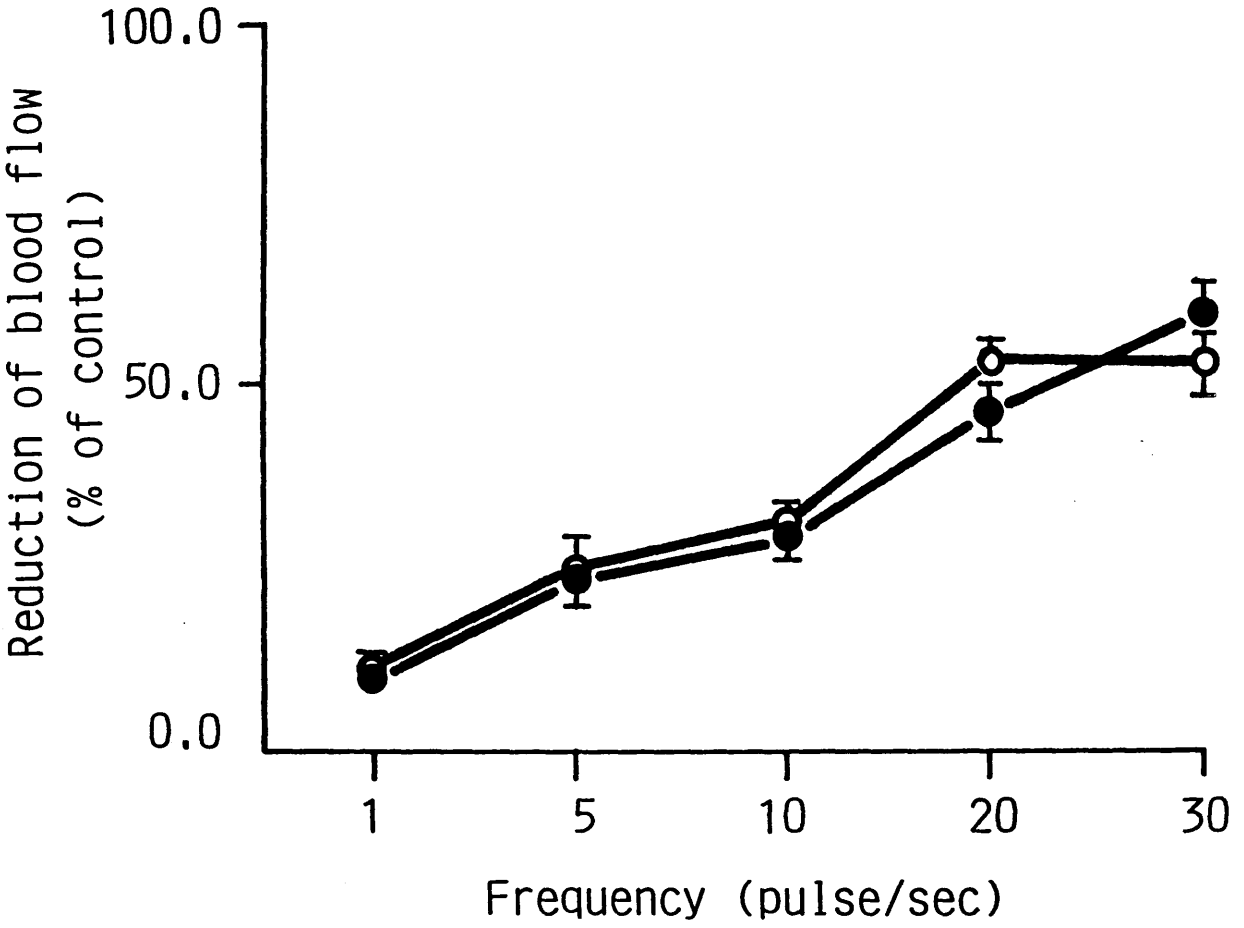

100.0
50.0
0.0
Reduction of blood flow
(% of control)
1
5
10
20
30
Frequency (pulse/sec)

Table 8

NO of animal	Date	Constrictor reponse	Dilator response	Dilator followed using α blocker
1.	15/5/88	+++	++	
2.	21/1/89	+++	-	
3.	27/1/89	++	-	
4.	1 /2/89	++++	-	
5.	8 /2/89	++++	-	
6.	12/2/89	+++	+++	
7.	15/2/89	+++	+*	+
8.	22/2/89	+++	+	+
9.	28/2/89	++	+*	+
10.	8 /3/89	++	+++	++
11.	15/3/89	+++	++	++
12.	19/4/89	++++	-	
13.	25/5/89	+++	+	
14.	3 /5/89	++	-	

Table 8: The responses of articular blood vessels to posterior articular nerve stimulation in 14 cats. (-); No response, (+); Up to 15%, (++); 15-30%, (+++); 30-50%, (++++); 50% and more, * Dilator response absent initially but appeared later in recording period.

considerable enhancement of constrictor response to increasing the frequency but little significant change to increasing the voltage. As illustrated in figure 5.22, there was a consistent response to PAN stimulation following at intervals of five minutes. Different trains of stimuli were also applied while the frequency and voltage were maintained constant at 10HZ and 10Volts respectively (Figure 5.23). As shown, the longer the duration of the stimulus train, the greater the constrictor response and the longer the recovery period.

c. The effect of different antagonists on PAN stimulation

The object of these experiments was to assess whether PAN stimulation mediates its constrictor effect via noradrenaline or other mediator(s) released from sympathetic efferent fibres. Figure 5.24 shows that the constrictor response was lowered following perfusion of knee joint vessels with guanethidine (10^{-5}M) which blocks the release of neurotransmitters such as noradrenaline, ATP, and NPY (Lundberg et al 1984). As shown in figures 5.25, 5.26, close intra-arterial injection of phentolamine, an α_1, α_2 -blocker(10^{-5}M); and prazosin, an α_1-blocker (10^{-5}M), like guanethidine, attenuated the constrictor response due to nerve stimulation but rauwolscine, an α_2 -blocker (10^{-5}M), and α, β mATP, a P_2-purinoceptor desensitiser(10^{-5}M) did not. It is noticeable that perfusion with guanethidine, and

Fig. 5.22. Upper trace shows consistent vasoconstrictor response 5 min after previous period of stimulation. S: time of stimulation. Arterial blood pressure (middle trace) and heart rate (lower trace) were also monitored.

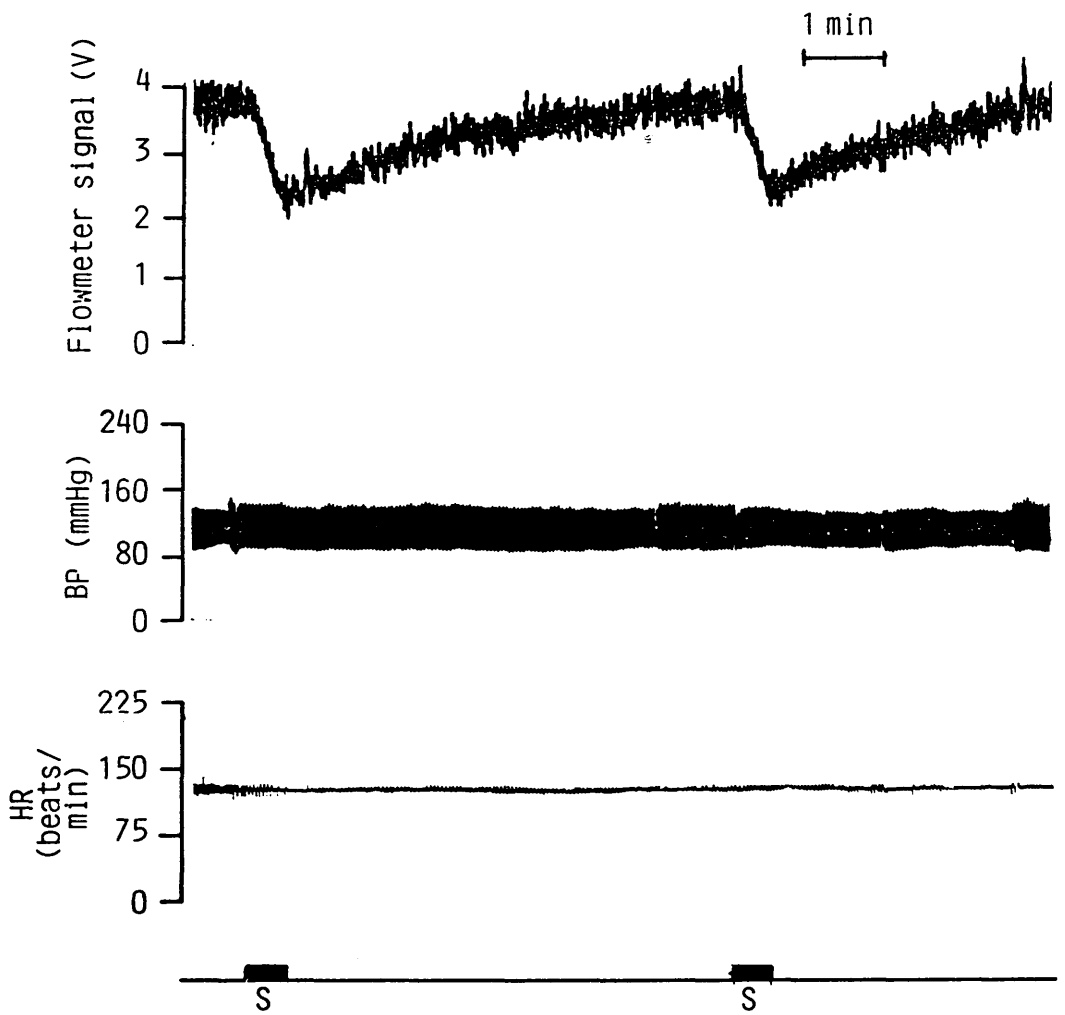

1 min
Flowmeter signal (V)
4
3
2
1
0
BP (mmHg)
240
160
80
0
HR (beats/min)
225
150
75
0
S
S

Fig. 5.23. The effect of PAN stimulation at different durations but at the same magnitude (10V), pulse width (1msec), and frequency (10HZ). The longer the duration of stimulus resulted in greater constrictor response and longer recovery period. Bars represent the period of stimulation. Arterial blood pressure (middle trace) and heart rate (lower trace) were also monitored. Since PAN was sectioned centrally, no changes in blood pressure or heart rate were observed during nerve stimulation.

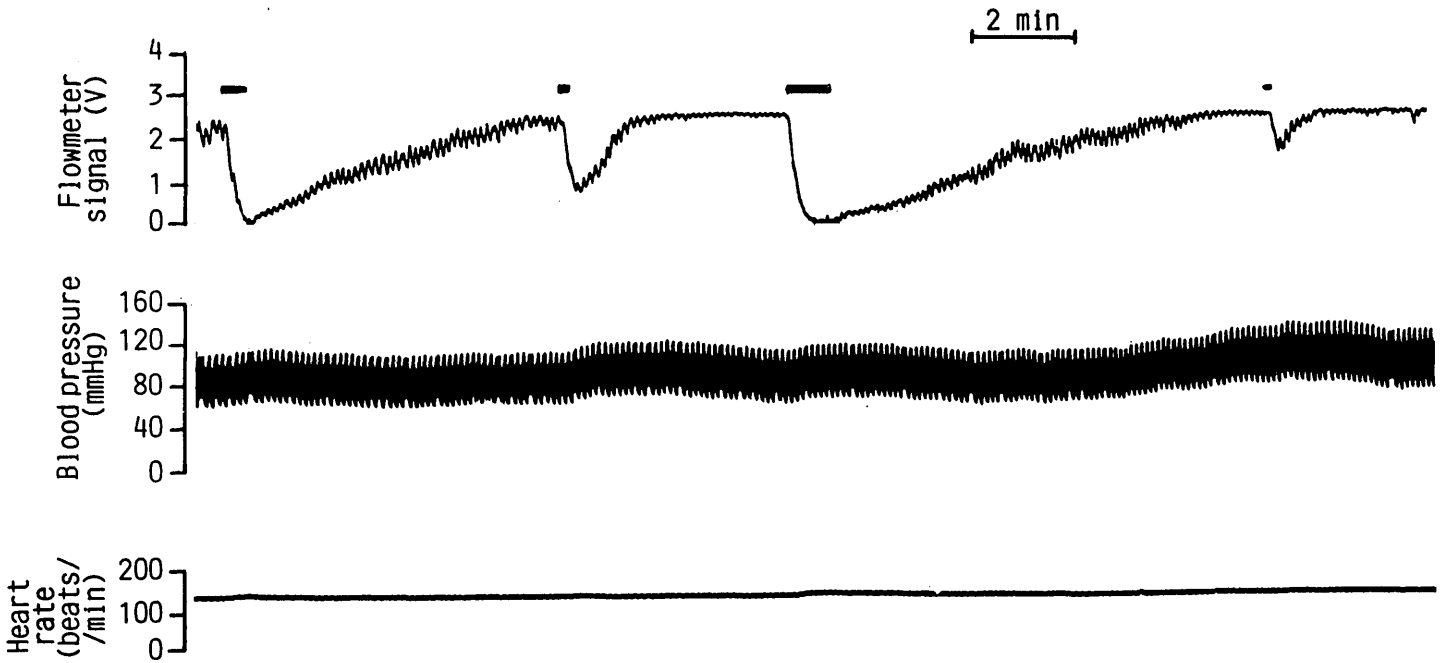

2 min
Flowmeter signal (V)
4
3
2
1
0
Blood pressure (mmHg)
160
120
80
40
0
Heart rate (beats/min)
200
100
0

Fig. 5.24. Upper trace show the effect of guanethidine (10^{-5}M) perfusion on vasoconstrictor responses of articular blood vessels. By removing the constrictor response, the dilator response to PAN stimulation became obvious. S bars represent periods of stimulation. Middle and lower traces show arterial blood pressure and heart rate changes respectively. A: start of guanethidine perfusion, B: perfusion stopped.

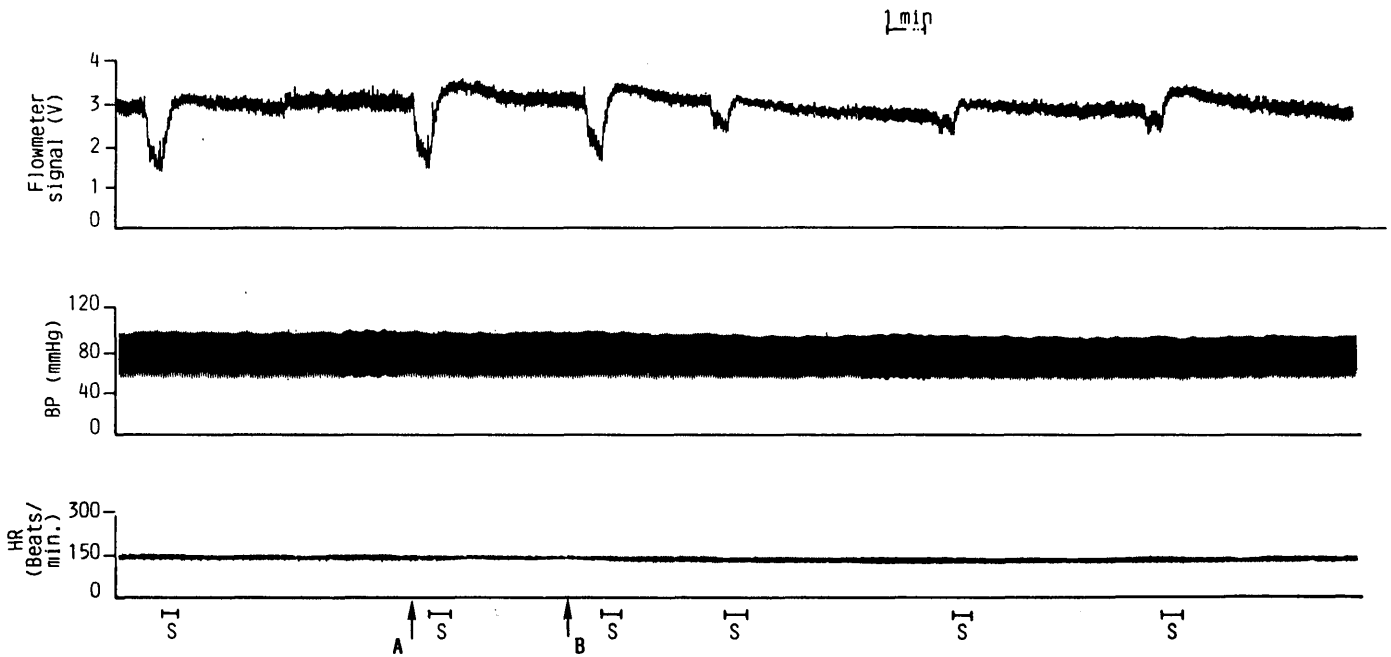

1 min
Flowmeter signal (V)
4
3
2
1
0
BP (mmHg)
120
80
40
0
HR (Beats/min.)
300
150
0
S
A
S
B
S
S
S
S

Fig. 5.25. The constrictor response to PAN stimulation is shown before (black histogram) and after administration of different antagonists, GUA; guanithidine (10^{-5}M), PHA; phentolamine (10^{-5}M) , PRA; prazosin (10^{-5}M), RAW; rauwolscine (10^{-5}M), and α, β mATP (10^{-5}M). n= 5 - 8, NS: Non significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

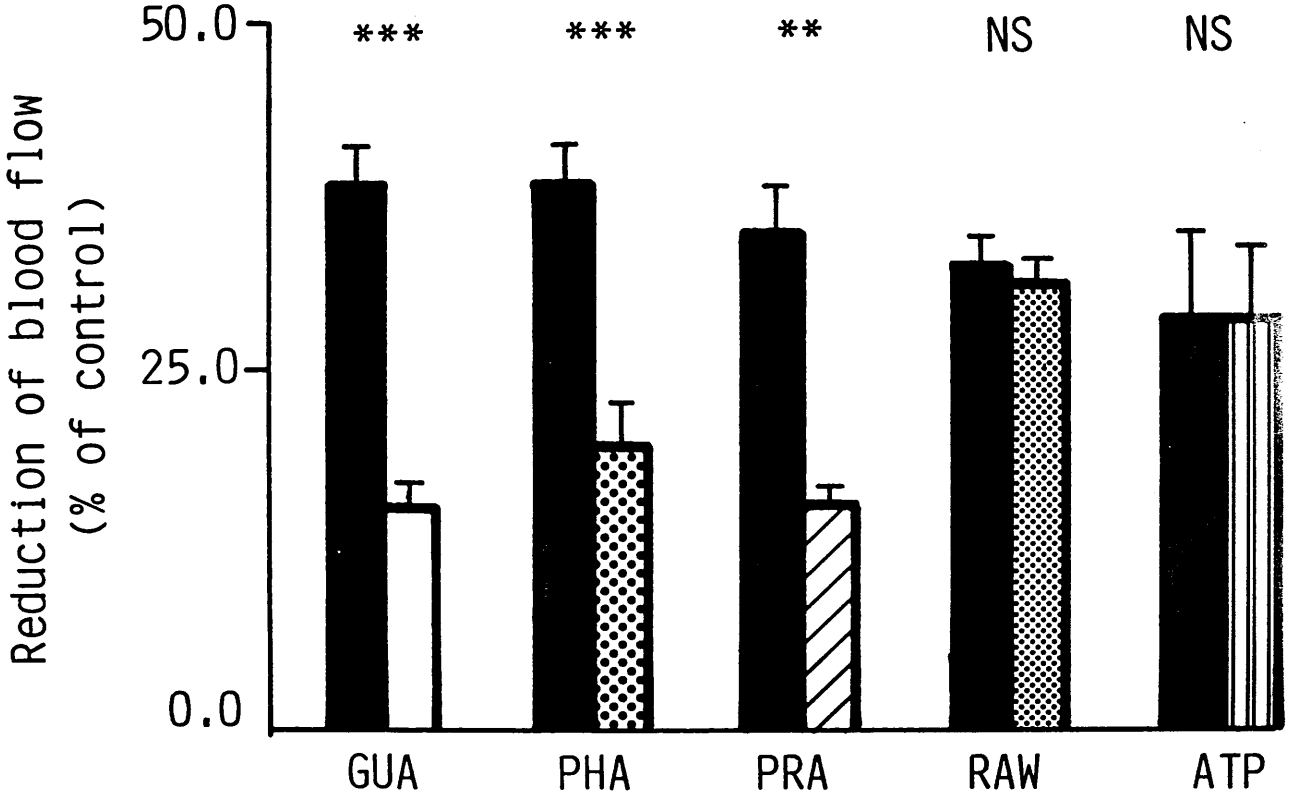
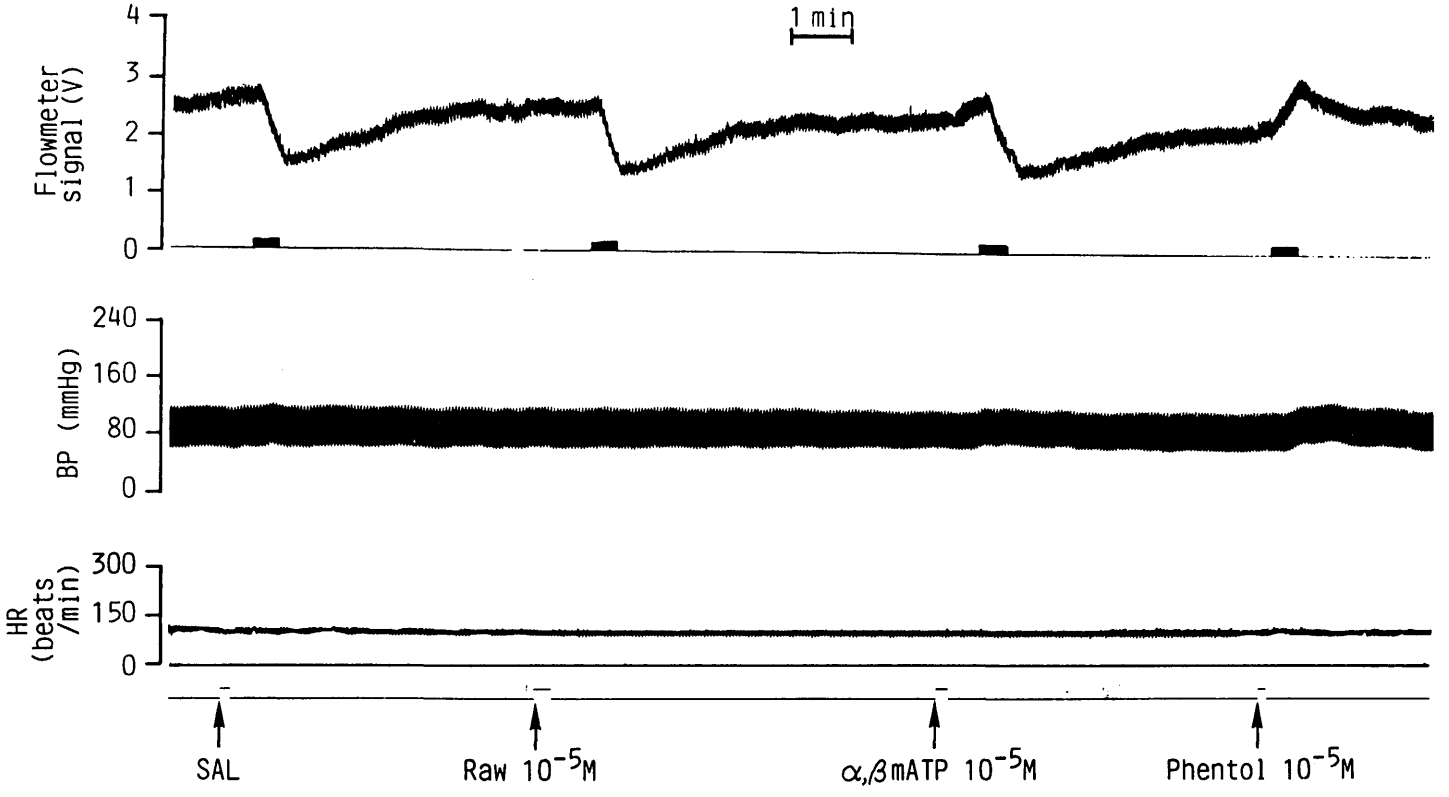

50.0
25.0
0.0
Reduction of blood flow
(% of control)
***
***
**
NS
NS
GUA
PHA
PRA
RAW
ATP

Fig. 5.26. PAN stimulation induces a vasoconstrictor response in articular blood vessels (10HZ, 10V, 1msec). This response was not affected by rauwolscine (RAW 10^{-5}M), or α, ß mATP (10^{-5}M), which were both administered intra-arterially prior to nerve stimulation. The vasoconstrictor response was not only abolished by administration of phentolamine (10^{-5}M), but a dilator response also became evident (upper trace). SAL: injection of 0.2ml saline. Middle and lower traces show arterial blood pressure and heart rate changes respectively.


1 min
Flowmeter signal (V)
4
3
2
1
0
BP (mmHg)
240
160
80
0
HR (beats /min)
300
150
0
SAL
Raw 10⁻⁵M
α,βmATP 10⁻⁵M
Phentol 10⁻⁵M

phentolamine not only attenuated the constrictor response to PAN stimulation but also it enhanced the dilator response (Figures 5.24, 5.26). The effectiveness of phentolamine and prazosin but not rauwolscine and α,β mATP in reducing the constrictor effect of PAN stimulation on articular blood vessels suggests that the neurotransmitter involved in constriction of these vessels is noradrenaline acting upon α_1-adrenoceptors.

d. Dorsal root stimulation

In early experiments antidromic activation of knee joint C fibre afferents was achieved by direct electrical stimulation of PAN (Ferrell and Cant 1987). To see whether the dilator response to PAN stimulation in these experiments was due to activation of afferent C-fibres, dorsal roots of L_7 and S_1 (which include most of afferent fibres arising from sensory receptors in the dorsal aspect of the knee joint capsule (Skoglund 1956)) were stimulated. As illustrated in figure 5.27, electrical stimulation of L_7 induced a monophasic dilator response in articular blood vessels. Comparing the dilator responses of L_7 and PAN stimulation together, the response to PAN stimulation was less (Figure 5.28). This could be because of activation of efferent sympathetic fibres, as the dilator response was significantly greater due to PAN stimulation when an α-blocker was used (Figure 5.28). The dilator response due to either L_7 , or PAN stimulation was reduced by intra-articular injection of 100ug of

Fig. 5.27. Traces of representative recordings of articular blood flow changes are shown (upper trace) when L_7 dorsal roots were stimulated (10HZ, 10V, 1msec)before (A) and after (B) administration of intra-articular injection of substance-P antagonist (100ug). There is no changes in either arterial blood pressure (middle trace) or heart rate (lower trace). Bars indicate time of stimulation.

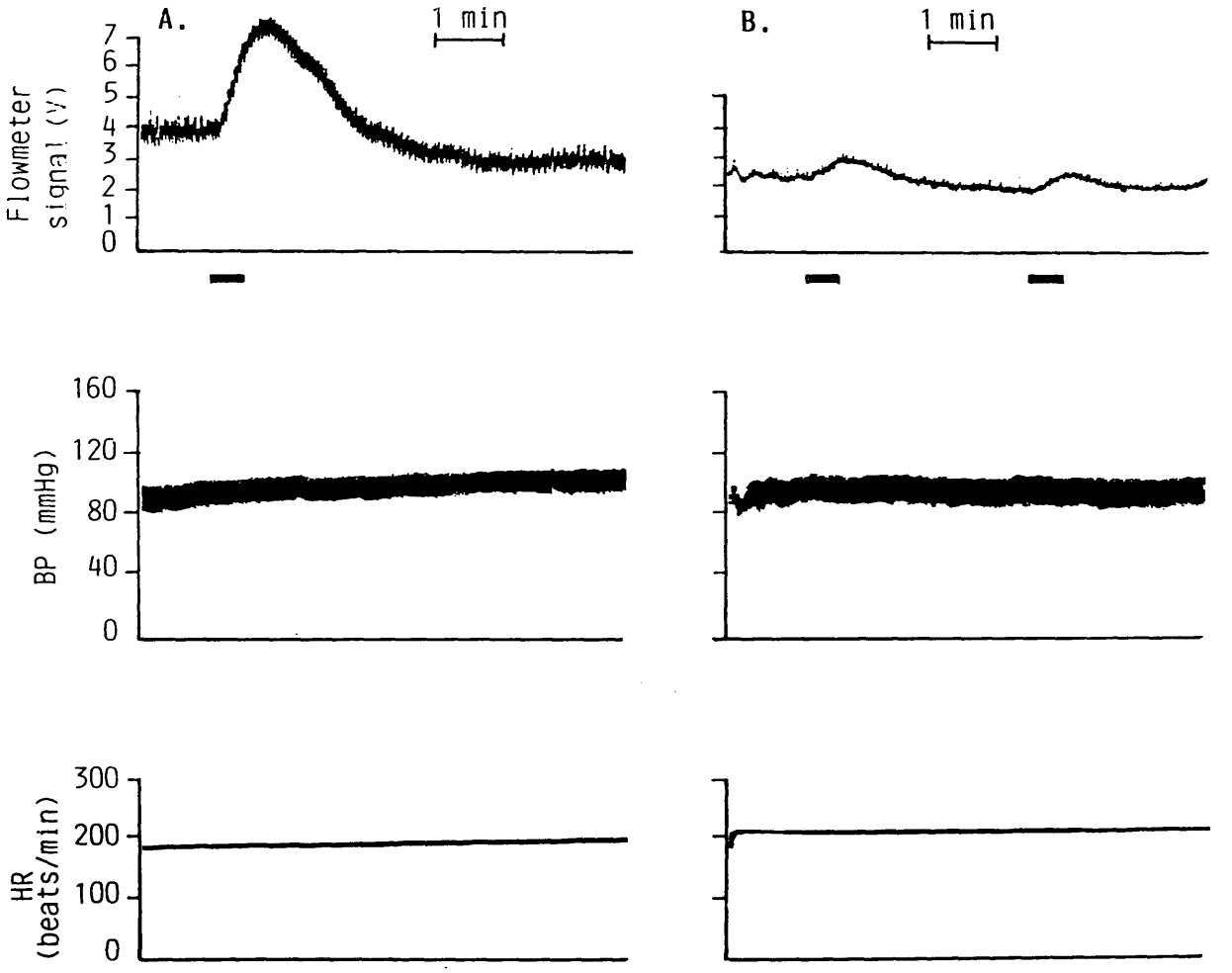
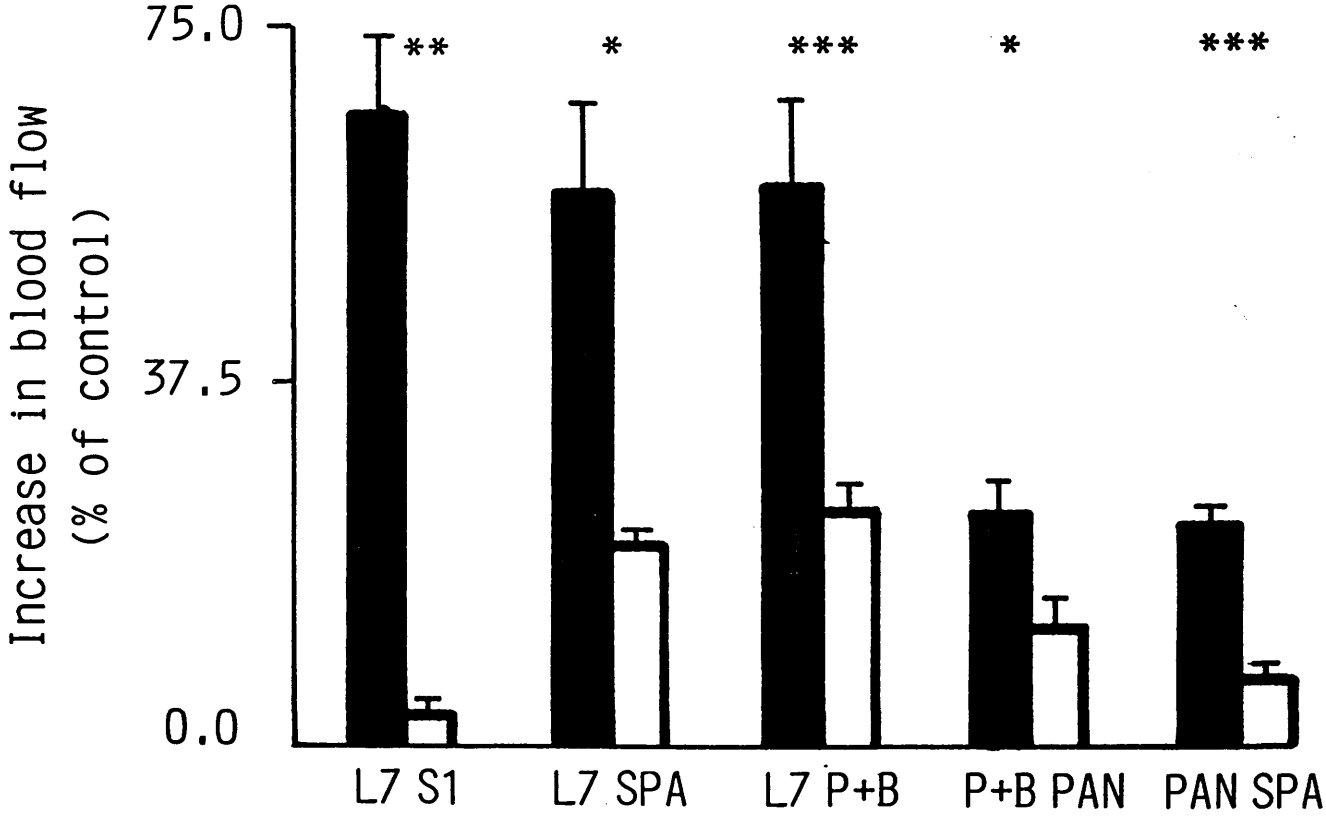

A.
1 min
B.
1 min
Flowmeter signal (V)
7
6
5
4
3
2
1
0
BP (mmHg)
160
120
80
40
0
HR (beats/min)
300
200
100
0

Fig. 5.28. Comparison of the dilator response to electrical stimulation of L_7 and S_1, L_7 stimulation before and after intra-articular injection of a substance P antagonist (SPA); L_7 and PAN stimulation after injection of an α blocker such as phentolamine (P+B); PAN stimulation before and after the α blocker (P+B), and PAN stimulation before and after SPA. As shown in this figure L_7 is the dorsal root which contains most of afferent C fibres arising from the posterior joint capsule. Responses to either L_7 and PAN stimulation were significantly reduced by SPA. n = 7-12 * $P<0.05$, ** $P<0.01$, *** $P<0.001$.


75.0
37.5
0.0
Increase in blood flow
(% of control)
**
*
***
*
***
L7 S1
L7 SPA
L7 P+B
P+B PAN
PAN SPA

substance P antagonist (D-Pro 4, D-Trp 7, 9, 10)- SP(4-11) prior to electrical stimulation of these nerves(Figures 5.27, 5.28).

DISCUSSION

The purposes of the present investigation were three fold; first, to attempt to identify response of articular blood vessels to exogenous drugs such as adrenaline (AD) isoprenaline (ISO), histamine (HIS), and acetylcholine (ACh) and quantify these responses. Second, to assess the changes in knee joint blood flow during stimulation of nerves supplying these vessels. Third, to identify the mediator(s) released during nerve stimulation.

1. The effect of AD, ISO, HIS, and ACh

The present data show that adrenaline and isoprenaline induce dose-dependent vasoconstriction in articular blood vessels. The reduction in the flowmeter signal during administration of ISO could have resulted from either fall in blood pressure or shunting of blood flow to surrounding muscles in addition to any articular blood vessel constriction. However, these are unlikely to have contributed significantly as the effect was attenuated by phentolamine but not by propanolol. Thus there is no evidence to indicate the presence of ß adrenoceptors on articular blood vessels in the cat. The constrictor effect was consistent with the findings of Cobbold and Lewis in dog (1956a), and Ferrell and Khoshbaten in rabbit (1989a). However, Cobbold and Lewis

did not quantify their results.

In this vascular bed, intra-arterial injection of low to high concentrations of ACh resulted in graded vasodilator response, with a fall in the arterial diastolic pressure. Fall in arterial diastolic pressure could be the effect of ACh on other blood vessels inducing vasodilation while the heart rate did not change. The relaxation of vascular smooth muscle by ACh was highly sensitive to blockade by atropine.

ACh released from sympathetic cholinergic nerves innervating the precapillary resistance vessels of skeletal muscles cause dilatation of these blood vessels acting directly on vascular smooth muscle cells (Berne and Levy 1986). Another possibility is that ACh also produces a powerful vasodilator action in-vivo via endothelial derived relaxant factor(EDRF) released from the endothelium (Furchgott 1981). This possibility was also confirmed in another study in rabbit knee joint blood vessels by Ferrell and Khoshbaten (1989b). Whether the effect of ACh on articular blood vessels in the cat is either a direct effect on vascular smooth muscle or via endothelial layer lining internal surface of these vessels requires further investigation.

In some cats, the effect of intra-arterial injection of histamine was tested. The results show an initial vasodilation followed by a prolonged constrictor effect in knee joint blood vessels. The constrictor effect was

dose-dependent.

Reports have indicated that histamine induces vasodilation in most blood vessels (Haddy 1960, Powell and Brody 1976) as well as increasing capillary permeability (Lewis and Grant 1924). However, in other studies, histamine was used to enhance the contractility of some vascular smooth muscles (Kennedy and Burnstock 1985, Furchgott 1981). Histamine acts on two separate and distinct receptors, termed H_1 and H_2 receptors. Both H_1 and H_2 receptors could mediate the vasodilator effect of histamine (Powell and Brody 1976). The H_1 receptors are generally more important, except for certain area such as temporal artery in human (Powell and Brody 1976). As these results show, both responses (dilator and constrictor effects) are present in articular blood vessels of cats, although the dilator reponse was absent in the same blood vessels in the rabbit (chapter 4, section III of this research). The possibility that fall in articular blood flow could be the result of dilatation of other adjacent vascular beds was ruled out, because at the same time that arterial blood pressure was returning to the baseline, the articular blood vessels were still constricted. It is also noticeable these effects were significantly reduced by injection of diphenhydramine (H_1-blocker), so it is suggested that most of histamine effect was via H_1 -receptors. But whether histamine exerts its effects via receptors on vascular smooth muscles or the endothelial layer or both is unclear.

2.The effect of nerve stimulation

Stimulation of posterior articular nerve resulted in an initial vasoconstriction of articular blood vessels of all animals (14 cats) during the stimulation period followed by a prolonged vasodilation on cessation of stimulation (in 8 cats). The results of the present investigation indicate that, consistent with the findings of Cobbold and Lewis in the dog (1956b), and Khoshbaten and Ferrell in the rabbit (1989), articular nerves (e.g. PAN), in the cat also contain sympathetic efferent fibres which are vasoconstricting in nature. The dilator response was observed in previous experiments carried out in the cat by Ferrell and Cant (1987) but not in the observations made by Cobbold and Lewis (1956b). The present observations more closely resemble those obtained by Ferrell and Cant (1987).

The dilator response was demonstrated to arise from afferent fibres as it could be elicited on electrical stimulation of the appropriate dorsal roots (L_7). The lack of dilator response in the experiments of Cobbold and Lewis (1956b) is perhaps due to differences in the techniques, species, or more likely differences in the nerve used (MAN vs PAN). The differences between present results where in some animals the dilator response was absent, and those of Ferrell and Cant (1987) could be due to differences in techniques of blood flow monitoring. In the present research more localised areas of the posterior

capsule were used by placing the tip of the probe on different areas of capsule. So it can also be due to differences in the distribution of afferent C fibres. In experiments where the dilator response was absent, the possibility exists that the prolonged recovery period followed vasoconstriction induced by PAN stimulation could mask the dilator response.

The findings from this study, taken together with the results of previous experiments (Ferrell and Russell 1985, Ferrell and Cant 1987, Khoshbaten and Ferrell 1989), indicate that the components of neurogenic inflammation -vasodilation and increased permeability of blood vessels- occur on antidromic stimulation of afferent C fibres in articular nerve. It is possible these fibres could contribute to the initiation or maintenance of the inflammatory process. Therefore, the potential for a neurogenic component of joint inflammation exists, particulary in view of the finding that the spontaneous activity of C fibres is enhanced in acutely inflamed joints in cat (Coggeshall et al 1983) and rats (Guilband et al 1985).

3. Mediator released during nerve stimulation

These results show that like other blood vessels, articular blood vessels are also innervated by sympathetic efferent fibres. The vasoconstrictor response to electrical nerve stimulation (PAN) was significantly

reduced almost equally effectively by the α_1, α_2 -blocker phentolamine, by guanithidine, and by the α_1 -blocker prazosin, suggesting this response is mainly mediated by noradrenaline. The vasoconstrictor response which was due to PAN stimulation in the cat appeared to be resistant to rauwolscine, an α_2 -adrenoceptor antagonist. These results are consistent with the previous observation in rabbit that NA released from sympathetic efferent fibres is acting mainly via α_1 -adrenoceptors (Ferrell and Khoshbaten 1989c).

There is compelling evidence that ATP is stored together with NA in adrenergic nerves (Su 1975 Levitt and Westfall 1982, Sneddon and Burnstock 1984, Burnstock 1985). In order to test this possibility that ATP is contributing as mediator or transmitter from articular sympathetic nerve endings, the effect of a P_2 -purinoceptor desensitiser, α, β methylene ATP was tried. α, β mATP did not change the magnitude of constrictor response. So in present study no evidence was obtained to indicate that ATP is released from nerve endings and contributes towards the vasoconstriction elicited by electrical stimulation of PAN.

Intra-articular injection of substance P antagonist reduced the magnitude of dilator response elicited by either electrical stimulation of dorsal roots (L_7) or PAN stimulation. Assuming that (D-Pro4,D-Trp7,9,10)-SP(4-11) is a specific substance P antagonist, it would appear

that the dilator response is mediated by articular afferents which release substance P from their terminals when depolarised. This is very similar to the results from the rabbit experiments (chapter 4 section II).

Therefore, by knowing the existence of substance P in the articular C fibre afferents, and their vasodilator effect, it is possible that these fibres may have an important role to play in local regulation of blood flow and also in acute inflammatory joint diseases besides their well-known role in nociception.

****

****

****

***

*

Appendix A

a. Preparation of Gelatin-KI-Barium Sulfate

All the procedures and ingredients which were used for preparation of Gelatin -KI- Barium Sulfate were carried out according to technique of Schlesingers medium (Schlesinger 1957).

b. Injection of specimen

A polythene cannula which has been already inserted into the popliteal artery was gently flushed with a few ml of saline solution to free articular blood vessels of air and to identify leaks so that they may be stopped. With the specimen ready for injection, procedures were:

(i). The bottle of the Gelatin - KI - Barium mass was shaken to distribute the barium sulfate evenly.

(ii). 26ml of the mass poured into small bottle.

(iii). 1ml distilled water was added to it.

(iv). 3ml formalin (40%) was also added.

(v). Immediately after adding of formalin, 1ml injection was administered by 1ml syringe.

(vi). After injection of radiopaque mass, the whole tissue was transferred to a bottle filled with formalin (10%) to be fixed.

c. Clearing technique

Following the tissue fixation in the formalin (10%) for a day, clearing method was carried out to identify the existence of radiopaque mass in the blood vessels.

Clearing method:

(i). Tissue was washed out with tap water.

(ii). Tissue was transferred to 70 percent alcohol (methyl alcohol) for 12 hours.

(iii). Tissue was again transferred to 90 percent alcohol for 24 hours and this was repeated for following seven days

(iv). For final dehydration, tissue was transferred to 100 percent alcohol (ethyl alcohol) for 24 hours and seven times.

(v). At last, for clearing, tissue was mounted into methyl sulicylate for about 4-6 hours.

(vi). When tissue was cleared some pictures were taken to visualized where the radiopaque mass were.

As it is shown in figure 2.14 radiopaque mass are found in the joint blood vessels. Although disadvantage of this method was that it can not show whether the radiopaque mass were in blood vessels supply bones or not, bu this possibility was ruled out by Evans blue injection technique.

Appendix B

Processing for semi-thin histology

1. The specimens were fixed in 10% formalin.
2. They were washed with:
   a. Millonig's buffer for 0.5 hour.
   b. 70% ethanol alcohol for 1 hour.
   c. 90% ethanol alcohol for 1 hour.
   d. 100% ethanol alcohol, four changes over six hours period.
   e. Propylene oxide, Two changes over 40 minutes.
   f. Propylene oxide/ Spurr's resin 1:1 mixture, for eight hours.
   g. Propylene oxide/ Spurr's resin 1:3 mixture, for eight hours.
   h. Pure Spurr's resin, twice over 48 hours.
3. Specimens were then embedded in fresh Spurr's resin. Polymerisation began overnight at 35 °C rising thereafter to 65 °C for 24 hours.
4. Serial sections were then cut on a reichert-jung autocut ultramicrotome at a thickness of 1 um, using 6mm wide glass knives of the Latta-Hartman type
5. Section were mounted on the glass slides and left to dry.
6. Sections were then stained either by Toludine blue or Haematoxyline and Eosin (H & E).

Process of H & E staining:

The sections have been:

1. Deresinated in saturated NaOH in Ethanol for 20mins.
2. Hydrated in:
   a. Absolute alcohol for 30sec - 1min, three times.
   b. 90% alcohol (Ethyl) 30sec - 1min.
   c. 70% alcohol (Ethyl) 1-2min.
3. Washed in water twice.
4. Stained with wiegert's Haemotoxyline for 5-7min.
5. Washed in water.
6. Differentiated in 1% acid/alcohol.
7. Washed in water.
8. Blued in Scotts.
9. Washed in water
10. Stained in 2% eosin for 10-20min.
11. Washed in water
12. Dehydrated in:
   a. 70% alcohol (Differentiated eosin).
   b. 90% alcohol (Differentiated eosin).
   c. Absolute alcohol twice.
13. Cleared in xylene for 5min.
14. Mounted in Histomount.

Process of Toludine blue

Sections have been:

1. stained with toludine blue for 10sec.
2. Dried with air
3. Mounted in Histomount.

REFERENCES

REFERENCES

Ahlquist, R.P. (1948) A study of adrenotropic receptors American Journal of Physiology. 153: 586-600.

Ahn, H., Lindhagen, J. ,Nillson, G.E. (1985). Evaluation of Laser Doppler flowmetry in the assessment of intestinal blood flow in cat. Gastroenterology. 88, 951-957.

Allen, J.M. ,Gjorstrup, P. ,Bjorkman, J.A. ,Er,L. , Abrahamson, T. ,and Bloom, S.R. (1986). Studies on cardiac distribution and function of NPY. Acta. Physiol. Scand. 126, 405.

Angelakos, E.T. (1965). regional distribution of catecholamines in the dog heart. Circ. Res. 16, 39-44.

Angelakos, E.T. ,Fuxe, K. ,and Torchiana, M.L. (1963). Chemical and histochemical evaluation of the distribution of catecholamines in the rabbit and guina-pig hearts. Acta. Physiol. Scand. 59, 184-192

Angelakos, E.T., Glassman, R.W., Millard, R.W.,and king,H.(1965). region distribution and subcellular localisation of catecholamines in the frog heart. Comp. Biochem. Physiol. 15,313-324.

Antontone, A.H., and Sayer, D.F. (1962). A study of

Aukland, K., Brower, B.F., Berliner, R.W.(1964). Measurement of local blood flow with hydrogen gas. Circ. Res. 14, 164-187.

factors affecting the alminium oxide trihydroxyindole procedure for the analysis of catecholamine. J. Pharmacol. Exp. Ther. 138, 360-375.

Augustinsson, K.B. (1948). Cholinestrases. A study in comprative enzymology.Acta. Physiol. Scand. 15(Suppl. 52).

Barger, G. and Dale, H.H. (1910). Chemical structure and sympathomimetic action of amines. J.Physiol. 41, 19-59.

Barone, R. ,Pavaux, C. Blin, P.C. ,Cuq, P. Atlas D' Anatomie. Du Lapin (Atlas of rabbit anatomy). Masson Et Cie Publisher.

Beck, L. and Brody, M.J.(1961). Physiology of vasodilation, Angiology. 12, 202-222.

Bell, C. (1968). Dual vasoconstrictor and vasodilator innervation of the uterine arterial supply in the guinea-pig. Cir. Res. 23, 279-289.

Berne M.B. ,Levy, M.N. (1981). Cardiovascular physiology, 4TH Ed. C. V. Mosby Company, pp123-144.

Bonner, R.F.,Clem, T.R.,Bowen, P.D. ,and Bowman, R.L. (1981). Laser Doppler continuous real-time monitor of pulsatile and mean blood flow in tissue microcirculation. In: Scattering Techniques Applied to Supramolecular and Non-Equilibrium System. edited by S.H. Chen, B.Chu, and R.

Buckberg, G., Luck,J.C., Payne,D.B., Hoffmann, J.E., Archie,J.P. and Fixler,D.E. (1971). Some sources of error in measuring regional blood flow with radioactive microspheres. J. Appl. Physiol. 31, 598-604.

Mossal. New York: Plenum, vol.73, p.685-702.(NATO ASI Ser.B).

Bonney, G.L.W. ,Hughes, R.A. ,and Janus, O. (1952). Blood flow through the normal human knee segment. Clin. Sci. 11, 167-181.

Bouteiller, G. ,Blasco, A. ,Decamps, J.L. et al (1980). Use of radiolabelled microspheres for simultaneous determination of total and regional blood flows in canine femure. Metab. Bone Dis. Relat. Res. 2, 123-129.

Brown, G.L., Gillespie, J.S. (1957). The output of sympathetic transmitter from the spleen of the cat. J.Physiol. 138, 81-102.

Brownstein, M.J., Saavedra, J.M. ,Axelrod, J. ,Zeman, G.H. ,and Carpenter, D.O. (1974). Co-existence of several putative neurotransmitters in single identified neurons of aplysia. Proc. Natn. Acad. Sci., USA 71, 4662-4665.

Bunge, R.,Johnson, M. ,and Ross, C.D. (1978) Nature and narture in developement of the autonomic neurone. Science. 199, 1409-1416.

Bunger, C. Hjermind, J. , Bach, P. Bunger, E.H. ,and Myhre-jenson, O. (1984). Haemodynamic in acute arthritis of the knee in puppies. Acta. Orthop. Scand. 55, 197-202.

Burnstock, G. (1970). Structure of smooth muscle and its innervation. in: Smooth muscle pp. 1-69 Eds. Bulbring, A. Brading, A. Jones & Tomita, T. Edward Arnold. London.

Burnstock, G. (1972). Purinergic nerves. Pharmacol. Rev. 24 (3), 509-581.

Bunger, C. ,Hjermind, J. ,Bulow, J. (1983). Haemodynamics of the juvinile knee in relation to increasing intra-articular pressure. An experimental study in dog. Acta. Orthop. Scand. 54, 80-87.

Burnstock, G. (1969) Evolution of the autonomic innervation of Visceral and Cardiovascular system in vertebrates Pharmacological Reviews. 21: 247-324

Burnstock, G. (1975).Control of smooth muscle activity in vessels by adrenergic nerves and circulating catecholamines. In: Smooth muscle pharmacology and physiology, vol. 50,Paris INSERM, 251-64

Burnstock, G. (1976): Do some nerve cells release more than one transmitter? Neuroscience 1: 239-248

Burnstock, G. (1978). A basis for distinguishing two types of purinergic receptor. In Cell membrane receptors for drugs and hormones; A multidisciplinary approach. ed. Straub. R. W. & Bolis, L. pp. 107-118. New York: Raven Press.

Burnstock, G. (1979). Past and current evidence for the purinergic nerve hypothesis. In: Baer, H.P. and Drummond, G.I. (Eds.). Physiological and regulatory functions of adenosine and adenine nucleotides, Raven press, New York. 3-32.

Burnstock, G. (1980). Cholinergic and purinergic regulation of blood vessels; Handbook of Physiology; Section 2: The Cardiovascular System; vol.II:, Vascular Smooth Muscle, in Bohr, Somlyo, Sparks, pp. 567-612, Bethesda: American Physiology Society.

Burnstock, G. (1981a). Neurotransmitters and tropic factors in the autonomic nervous system. J. Physiol. 313, 1-35.

Burnstock, G. (1981b). Purinergic receptors. Receptors and recognition, series B, vol. 12. Chapman & Hall, London.

Burnstock, G. (1982a). A comparison of receptors for adenosine and adenine nucleotide. In Regulatory Function of Adenosine ed. Berne, R.M., Rall, T.W. & Rubio, R. pp. 49-62. The Hague: Martinus Nijhoff.

Burnstock, G. (1982b). The co-transmitter hypothesis, with special reference to the storage and release of ATP with noradrenaline and acetylcholine, in co-transmission, Ed: A. C. Cuello. Macmillan, London. pp151-163

Burnstock, G. (1983). Recent concepts of chemical communication between excitable cells. In: Osborne, N.N. (Ed.). Dales Principle and Communication between neurones, Pergamon press, Oxford. pp. 7-35.

Burnstock, G. (1985a).Nervous control of smooth muscle by transmitters, cotransmitters and modulators. Experimentia. 41, 869-874.

Burnstock, G. (1985b).Neurogenic control of cerebral circulation. Cephalagia. Suppl. 2, 25-33.

Burnstock G. (1986a). The non-adrenergic, non-cholinergic nervous system. Archives of International Pharmacodynamics 280: suppl. 1-15.

Burnstock G. (1986b). The changing face of autonomic neurotransmission. Acta Physiologica Scandinavica 126: 67-91.

Burnstock, G. & Brown, C.M. (1981). An introduction to purinergic receptors. In purinergic receptors (Receptor and Recognition, Series B. vol 12). ed. Burnstock, G. pp. 1-45. London: Chapman and Hall.

Burnstock, G. ,Campbell, G. ,Bennett, M. and Holman, M.E. (1963). Inhibition of smooth muscle of the taenia coli. Nature. 200, 581-582.

Burnstock, G. ,Campbell, G. ,Bennett, M. ,and Holman, M.E.(1964). Innervation of the guinea-pig taenia coli: are there intrinsic inhibitory nerves which are distinct from sympathetic nerves? Int. J. Neuropharmacol. 3, 163-166.

Burnstock, G., Campbell, G., Statchell, D. & Smythe, A. (1970) Evidence that adenosine triphosphate or a related nucleotides is the transmitter substance released by a non-adrenergic inhibitory nerves in the gut. British Journal of Pharmacology 40: 668-688.

Burnstock, G. ,Chamley, J. ,and Campbell, G.R. (1986). The innervation of arteries. In:Schwartz C.J., Werthessen, N.T., Wolf, S. eds:Structure and function of the circulation, vol. 1.New York, Plenum press, 729-767.

Burnstock, G., Griffith, S.G. & Sneddon, P. (1984). Autonomic nerves in the precapillary vessel wall. J. Cardiovasc. Pharmacol. 6: Suppl. 344-353.

Burnstock, G.& Kennedy,C. (1986). A dual function for Adenosine 5'-triphosphate in the regulation of vascular tone. Circ. Res. 58(3): 319-330.

Burnstock, G. and Sneddon, P. (1985) Evidence for ATP and noradrenaline as co-transmitters in sympathetic nerves Clinical Science 68 (suppl,10) 89S-92S.

Busija, D.W. ,Heistad, D.D. ,and Marcus, M.L. (1981). Continuous measurement of cerebral blood flow in anaesthetized cats and dogs. Am. J. Physiol. 241, H228-H234.

Byfield,R.A., Swayne, G.T.G. & Warner, T.J. (1986). A

method for the study of endothelial derived relaxing factor (EDRF) in the isolated perfused rat mesentery. Br. J. Pharmacol. 88: 438P.

Calvino, B. ,Vilanueva, L. ,and LeBars, D.(1987). Dorsal horn (convergent) neurones in the intact anaesthetized arthritic rat. I. segmental excitatory influences. Pain 28(1), 93-99.

Cambridge, D., Davey, M. J. and Massingham, R. (1977). Prazosin: A selective antagonist of post synaptic alpha-adrenoceptors. British J. of Pharmacology. 59, 514-515.

Christensen, S.B. ,Reimann, I. ,Henriksen, O. ,and Arnoldi, C.C. (1982). Experimental osteoarthritis in the rabbit. A study of ^{133}Xenon washout rates from the synovial cavity. Acta. Orthop. Scand. 53, 167-174.

Cobbold, A.F. & Lewis, O.J. (1956a). The action of adrenaline and noradrenaline and Ach on blood flow through joints. J. Physiol. 133: 472-474.

Cobbold A.F. & Lewis O.J. (1956b). The nervous control of joint blood vessels. Journal of Physiology 133: 467-471.

Cobbold A.F. & Lewis O.J. (1956c).Blood flow to the knee joint of the dog. Effect of heating, cooling and adrenaline. J. Physiol. 132, 379-383.

Couture, R. ,Cuello, A.C. (1984). Studies on the trigeminal antidromic vasodilation and plasma extravasation in the rat. J. Physiol. (London) 346, 273-286.

Coggeshall, R.E. ,Hong, K.a.l. ,Langford, L.A. Schaible, H.G. ,Shmidt, R.F.(1983). Discharge characteristics of fine medial articular afferents at rest and during passive movements of inflamed knee joints. Brain Res. 272, 185-188.

Cotsell, B. ,Foreman, J. ,Lynn, B. ,Shakhanbeh, J. ,and Ye, W. (1989). The use of an infra-red laser-Doppler flow meter to measure changes in skin blood flow in man and in experimental animals. University college London meeting Physiological society, D.16.

Dale, H.H. (1933). Nomenclature of fibres in the autonomic system and their effect. J. Physiol. (London) 80, 10-11.

Dardick, S.J. ,Basbaum, A.I. ,Levine, J.D. (1986). The contribution of pain to disability in experimentally induced arthritis. Arthritis Rheum. 29(8), 1017-1022.

DeMay, J., Burnstock, G., and Vanhoutte, P.M. (1979) Modulation of the evoked release of noradrenaline in canine saphenous vein via presynaptic receptors for adenosine but not ATP. European Journal of Pharmacology 55: 401-405.

De May,J.G. & Vanhoutte,P.M. (1981). Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. J. Physiol. 316: 347-355.

De May,J.G. & Vanhoutte,P.M. (1982). Heterogeneous behaviour of the canine arterial and venous wall: importance of endothelium. Circ. Res. 51: 439-447.

De May, J.G. & Vanhoutte,P.M. (1983). Anoxia and endothelium-dependent reactivity of the canine femoral artery. J. Physiol. 335, 65-74.

Dick, W.C. ,St. Onge R.A. ,Gillespie, F.C. ,Downie, W.W. , Nuke, G. ,Gordon, I. ,Whaley, K. ,Boyle, J.A. ,and Buchanan, W.W. (1970). Derivation of knee joint synovial perfusion using the xenon (^{133}Xe) clearance technique. Ann. Rheum. Dis. 29, 131-134.

Dick, W.C. ,Jubb, R. ,Buchanan, W.W. ,Williamson, J. Whaley, K. and Poter, B.B. (1971). Studies on the sympathetic control of normal and diseased synovial blood vessels: The effect of α and ß receptor stimulation and inhibition, monitored by the ^{133}Xenon clearance technique. Clin. Sci. 40, 197-209.

Docherty, J.R. ,Macdonald, A.and McGrath, J.C. (1979). Further sub classification of alpha-adrenoceptors in the C.V.S, vasdeferns and anococcygeus of the rat. British J. of Pharmacol. 67, 421-422.

Docherty, J.R. and McGrath, J.C. (1980). The factors influencing the time course of drug action at clonidine in

the pitted rat. British J. Pharmacol. 68, 225-234.

Edninsson, L.,Emson, P. ,Mcculloch, J. ,Tatemoto, K. ,and Uddman, R. (1983). Neuropeptide Y: Cerebro-vascular innervation and vasomotor effects in the cat. Neurosci. Lett. 43, 79.

Eliasson, S. (1952). Cerebral influence on gastric motility in the cat. Acta. Physiol. Scand. 26, suppl. 95, 1-70.

Eliasson, S. (1954). Activation of gastric motility from the brainstem of the cat. Acta. Physiol. Scand. 30, 199-214.

Elliot, T.R. (1905). The action of adrenaline J.Physiol. 32, 401-467.

Engelhart, M. and Kristensen, J.K. (1983). Evaluation of cutaneous blood flow responses by ^{133}Xenon washout and a Laser-Doppler flowmeter. J. of Invest. Dermatol. 80(1), 12-15.

Euler, U.S. von (1963). Adrenergic neurohormones. In Comparative Endocrinology ed. by U.S. von Euler and H. Heller, vol. 2, Academic press, New York, London.

Eyre, J.A., Essex,A.t., Flecknell, P.A., Bartholomew, P.H. and Sinclair, J.I. (1988). A comparison of measurements

of cerebral blood flow in rabbit using laser Doppler spectroscopy and radionuclide labbeled microspheres. Cli. Phys. Physiol. Meas. 9, 65-74.

Feld, A.D. ,Fondacaro, J.D. ,Holloway, G.A. Jr. ,andJacobson, E.D. (1984). Laser Doppler velocimetry: A new technique for the measurement of intestinal mucosal blood flow. Gastrointest. Endosc. 30, 225-230.

Feldberg, W. (1945). Present views on the mode of action of acetylcholine in the central nervous system. Physiol. Rev. 25, 596-642.

Ferrell W.R. & Cant R. (1987). Vasodilation of articular blood vessels induced by antidromic stimulation of articular C fibre afferents. In: Fine afferent fibres and pain eds. Schmidt R.F., Schaible H-G & Vahle-Hinz C. VCH, Weinheim.

Ferrell, W.R. & Khoshbaten, A. (1989a). Adrenoceptor profile of blood vessels in the knee joint of the rabbit. J. Physiol. 414, 377-383.

Ferrell, W.R. & Khoshbaten, A. (1989b). The role of the endothelium in mediating the actions of ATP, Adenosine and Acetylcholine on flow through blood vessels in the rabbit knee joint. British J. Pharmacol. (in press).

Ferrell, W.R. & Khoshbaten, A. (1989c). Responses of

Fitzgerald, M. (1989). Arthritis and the nervous system. TINS. 12, (3), 86-87.

blood vessels in the rabbit knee to electrical stimulation of the joint capsule. J. Physiol. (in press).

Ferrell, W.R. and Russell, N.J.W. (1985). Plasma extravasation in the cat knee joint induced by antidromic articular nerve stimulation. Pflugers Arch. 404, 91-93.

Fieschi, C. Bozzao, L. ,and Agnoli, A. (1965). Regional clearance of hydrogen as measure of cerebral blood flow. Acta. Neurol. Scand. Suppl. 14, 46-52.

Folkow, B. (1955). Nervous control of blood vessels. Physiol. Rev. 35, 629-663.

Folkow, B. , Mellander, S. and Oberg, B. (1961). The range of the effect of sympathetic vasodilator fibres with regard to consecutive sections of the muscle vessels. Acta. Physiol. Scand. 53, 7-22.

Frank, G.W. & Bevan, J.A. (1983). Vasodilation by adenosine-related nucleotides is reduced after endothelial destruction in basilar, lingual and pulmonary arteries; in Berne, R.M. Rall, T.W., & Rubio, R. Regulatory function of adenosine, pp. 511-512 (Nijhoff, the Hague).

Freeman, M.A.R. ,Wyke, B. (1967). The innervation of the knee joint. Anatomical and histological in cat. J. Anat. 101, 505-532.

Furchgott, R.F. (1972). The classification of adrenoceptors (adrenergic receptor): An evaluation from the standpoint of receptor theory. In handbook of experimental pharmacology. 33 Eds. Blashko, H. and Mascholl, E. Springer- verlag: Berlin.

Furchgott, R.F. (1981). The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. Trends Pharmacol. Sci. 2: 173-176.

Furchgott, R.F. & Zawadski, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373-376.

Furshpan, E.J. ,MacLeish, P.R. ,O'Lague, P.H., and Potter, D.D. (1976). Chemical transmission between rat sympathetic neurones and cardiac myocytes development in microcultures-evidence for cholinergic, adrenergic and dual function neurones. Pric. natn. Acad. Sci. USA. 73, 4225-4229.

Gardner, E.D. (1944). The distribution and termination of nerves in the knee joint of the cat. J. Comp. Neurol. 80, 11-32.

Gardner, E. (1950) Physiology of movable joints Physiological Reviews 30: 127-176.

Goth, A. (1981). Medical pharmacology. The C.V. Mosby co. London, pp. 115-135.

Gaskell, W.H. (1900). In Text book of physiology vol. 2, p.169. Edinburgh, London.

Geborek P., Forslind K. & Wollheim F.A.(1989) Direct assessment of synovial blood flow and its relation to induced hydrostatic pressure changes. Ann. Rheum.Dis. 48: 281-286.

Gillespie, J.S. (1982). Non-adrenergic, noncholinergic inhibitory control of gastrointestinal motility. In: Wienbeck, M. (Ed.). Motility of the Digestive Tract, Raven press, New York. pp.51-66.

Goldbeng, L.I. (1974). Dopamine-clinical uses of an endogenous catecholamine. N. Engl. J. Med. 291, 707.

Goodall, McCh. (1951). Studies of adrenaline and noradrenaline in mammalian heart and suprarenals. Acta. Phsiol. Scand. 24 (suppl. 85).

Goodrich, E.S. (1930). Studies of the structure and development of vertebrates. The Macmillan Company, London.

Gordon, J.L. & Martin, W. (1983). Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of ^{86}Rb efflux from isolated endothelial cells. Br. J. Pharmacol. 79: 531-541.

Greeff, K. ,Kasperat, H. and Osswald, W. (1962). Paradoxe

Green, J.F.(1982).Fundamental Cardiovascular and Pulmonary Physiology, An Integrated Approach for Medicine. Lea & Febiger co. U.S.A. pp. 144-158.

wirkungen der elektrischen am isolierten Magen-und Herzvorhofprapaparat des Meerschweinchens sowie deren Beeinflussung durch Ganglien blocker, Sympathicolytica, Reserpin und Cocain. Naunyn-Schmeidebergs Arch. Pharmackol. Exp. Patol. 243, 528-545.

Green, H.D. and Kepchar, J.H. (1959). Control of peripheral resistance in major systemic vascular beds. Physiol. Rev. 39,617.

Gross, P.L. ,Marcus, M.L. ,Heistad, D.D. (1981). Current concept review: Measurement of blood flow to bone and marrow in experimental animals by means of the microsphere technique. J. of Bone and Joint Surgery. 63-A, (6), 1028-1031.

Gross, P.L. ,Heistad, D.D. Marcus, M.L. (1979). Neurohumoral regulation of blood flow to bone and marrow. Am. J. Physiol. 237, H440-H448.

Grundfest,H. (1957). The mechanisms of discharge of the electric organs in relation to general and comparative electrophysiology. Progr. Biophys. Biophys. Chem. 7, 1-85.

Guilbaud, G. ,Iggo, A. ,Tegner, R. (1985). Sensory receptors in ankle joint capsules of normal and arthritic rats. Exp Brain Res. 58, 29-40.

Haberl, R.L. , Heizer, M.L. ,Marmarou, A. ,and Ellis, E.F.

(1989a). Laser-Doppler assessment of brain microcirculation: effect of systemic alterations. Am. J. Physiol. 256, H1247-H1254.

Haberl, R.L. , Heizer, M.L. ,and Ellis, E.F. (1989b). Laser Doppler assesment of brain microcirculation: Effect of local alterations. Am. J. Physiol. 256, (Heart Circ. Physiol. 25), H1255-H1260.

Haddy, F.(1960). Effect of histamine on small and large vessel pressures in the dog foreleg, Am, J. Physiol. 198, 161.

Haddy, F.J. & Scott, J.B. (1968). Metabolically linked vasoactive chemicals in local regulation of blood flow. Physiol. Rev. 48: 688-707.

Harris, R. and Millard, J. B. (1956). Clearance of radioactive sodium from the knee joint. Clin. Sci. 15, 9-15.

Harris, R. ,Millard, J.B. ,and Banerjee, S.K. (1958). Radiosodium clearance from the knee joint in rheumatoid arthritis. Ann. Rheum. Dis. 17, 189-195.

Head, R.J., Stitzel, R.E., Delaland, I.S. & Johnson, S.M.(1977): Effect of chronic denervation on activities of monoamino-oxidase and catechol-o-methyl transferase and on contents of NA and adenosine-triphosphate in rabbit ear

artery. Blood Vessels 14: 229-239

Hedlund, H., Fandriks, L., Delbro, D. & Fasth, S. (1983). Blockade of non-cholinergic, nonadrenergic colonic contraction in response to pelvic nerve stimulation by large doses ofα ,ß -methylene ATP. Acta Physiol. Scand. 119: 451-454.

Hellem, S. ,Jacobson, L.S. ,Nilsson, G.E. ,Lewis, D.H. (1983). Measurement of microvascular blood flow in cancellous bone using laser Doppler flowmetry and ^{133}Xe clearance. Int. J. Oral Surg. 165-177.

Hertzman, A.B. (1959). Vasomotor regulation of cutaneous circulation. Physiol. Rev. 39, 280-306.

Hesser, F.H. ,Perret, G.E. (1960). Studies on gastric motility in cat. II. Cerebral and infracerebral influence in control, vagotomy and cervical cord preparations. Gastroenterology. 38, 231-246.

Hicks, P.E., Langer, S.Z. and Vidal, M.J. (1985). α,ß-methylene ATP inhibits the vasoconstriction to peripheral field stimulation in SHR but not WKY tail arteries in-vitro. Brit. J. Pharmacol. 85, 225p.

Hirst, G.D.S. & Neild, T.O. (1980). Evidence for two populations of excitatory receptors for noradrenaline on the arteriolar smooth muscle Nature 283: 767-768.

Hunter, J. Whillans, M.G. (1951). A study of the effect of cold on joint temperature and mobility. Canad. J. Med. Sci. 29,255-262.

Hokfelt, T. (1951). Noradrenaline and adrenaline in mammalian tissue. Acta Physiol. Scand. 25 (suppl. 92), 1-134.

Holloway, G.A. ,and Watkins, D.W. (1977). Laser Doppler measurement of cutaneous blood flow. J. of Invest. Dermatol. 69, 306-309.

Holton, P. and Rand, M.J. (1962). Sympathetic vasodilation in the rabbit ear. British J. Pharmacol. Chemother. 19, 513-526.

Holtz, P. ,Kroneberg, G. ,and Schumann, H.J. (1951). Uber die sympathikomimetische wirksamkeit von Herzmuskelextrakten. Arch. exper. Pathol. Pharmakol. 212, 551-567.

Horvath, S.M. and Hollander, J.L. (1949). Intra-articular temperature as a measure of joint reaction. J. Clin. Invest. 28, 469-473.

Hubbard,J.I. and Quastel, D.M.J.(1973). micripharmacology of vertebrate neuro-muscular transmission. Annu. Rev. Pharmacol. 13, 199.

Kane, W.J. (1968). Fundamental concepts in bone blood flow studies. J. Bone and Joint Surgery. 50-A, 801-811.

Kasakov, L. & Burnstock, G. (1983). The use of the slowly degradable analog, α , ß -methylene ATP, to produce desensitisation of the P_2-Purinoceptor: effect on nonadrenergic, noncholinergic responses of the guina-pig urinary bladder. Eur. J. Pharmacol. 86: 291-294.

Kellgren, J.H. and Samuel, E.P. (1950). The sensitivity and innervation of the articular capsule. J. Bone and Joint Surgery. 32B, 84-92.

Kennedy, C. & Burnstock, G. (1985). ATP produces vasodilation via P_1-Purinoceptors and vasoconstriction via P_2-Purinoceptors in the isolated rabbit central ear artery. Blood Vessels 22: 145-155.

Kennedy, C., Delbro, D. & Burnstock, G. (1985). P_2-Purinoceptors mediate both vasodilation (via the endothelium) and vasoconstriction of the isolated rat femoral artery. Eur. J. Pharmacol. 107: 161-168.

Ketty, S.S. (1949). Measurement of regional circulation by the local clearance of radioactive sodium. Am. Heart J. 38, 321-328.

Khoshbaten, A. and Ferrell, W.R. (1989). Responses of blood vessels in the rabbit knee to acute joint inflammation. Annal. Rheu. Dis. (in press).

Kirchhoff, C.G. ,Reeh, P.W. ,Waddell, P.J. (1989).

Cutaneous sensory endings of C- and A-fibres are differentially sensitive to tetrodotoxin in the anaesthetized rat. Cambridge Meeting 21-22 july. C.112.

Knight A.D. & Levick J.R. (1983). The density and distribution of capillaries around a synovial cavity. Quarterly J. of Exp. Physiol. 68, 629-644.

Knight A.D. & Levick J.R. (1984). The influence of blood pressure on trans-synovial flow. Journal of Physiology 349: 27-42.

Kvietys, P.R., Shepherd, A.P. ,and Granger, D.N. (1985). Laser Doppler, H_2 clearance, and microsphere estimates of mucosal blood flow. Am. J. Physiol. 249, G221-G227.

Lam F.Y. & Ferrell W.R.(1989). Inhibition of carrageenan-induced joint inflammation by substance P antagonist. Ann. Rheum. Dis. (in press).

Landis, C.A. ,Robinson, C.R. ,and Levine, J.D. (1988). Sleep fragmentation in the arthritic rat. Pain. 34(1), 93-99.

Lands,A.M. ,Arnold, A.,and McAnliff, J.P. (1967). Differentiation of receptor systems activated by sympathomimetic amines. Nature. 214,597-598.

Langer, S. Z. (1974).Commentary: Presynaptic regulation of

catecholamine release. Biochem. Pharmacol. 23, 1793-1800.

Langer, S.Z.,& Pinto, J.E.B. (1976). Possible involvement of a transmitter different from norepinephrine in the residual response to nerve stimulation of the cat nictitating membrane after pretreatment with reserpine. Journal of Pharmacology and Experimental Therapeutics 196: 697-713.

Langford, L.A. (1983). Unmyelinated axon ratios in cat motor, cutaneous and articular nerves. Neurosci. Let. 40, 19-22.

Langford, L.A. ,and Schmidt, R.F.(1983). Afferent and efferent axons in the medial and posterior articular nerves of the cat. Anat Rec. 206, 71-78

Langley, J.N.(1878). On the mutual antagonism of atropin and pilocarpin. having especial refernce to their relations in the submaxillary gland of the cat. J.Physiol. 1, 339.

Langley, J.N.(1898). On inhibitory fibres in the vagus to the end of oesophague and the stomach. J.Physiol. 23,407-414.

Langley, J.N. (1905). On the reaction of cells and of nerve endings to certain poisons. Chiefly as regards the reaction of striated muscle to nicotine and to curare.

J.Physiol. 33, 374-413.

Langley,J.N. and Anderson, H.K.(1895). The innervation of the pelvic and adjoining vicera, IV. The internal generative organs. J. Physiol. 19,122-130.

Leduc, J. (1961). Catecholamine production and release in exposure and acclimation to cold. Acta. Physiol. Scand. 53, (suppl. 183) 1-92.

Levick J.R. (1984). Blood flow and mass transport in synovial joints. In Handbook of Physiology, The Cardiovascular System, vol. iv, The Microcirculation, ed Renkin E.M. & Michel C.C., pp. 917-947. Bethseda: American Physiological Society.

Levick J.R. (1987). Synovial fluid and trans-synovial flow in stationary and moving normal joints. Joint Loading, In: Biology and health of articular structures. Ed. by Helminen, H.J. ,Kiviranta,I.,Saamanen, A.M. ,Tammi, M. Paukkonen, K. ,Jurvelin, J. WRIGHT Bristol, pp. 149-186.

Levine, J.D. ,Dardick, S.J. ,Roizen, M.F. ,Helms,C. ,and Basbaum, A.I. (1986). Contribution of sensory afferents and sympathetic efferents to joint injury in experimental arthritis. J.Neurosci. 6, 3423-3429.

Levine, J.D. ,Coderre, T.J. ,Helms, C. ,and Basbaum, A.I. (1988). beta$_2$-adrenergic mechanisms in experimental

arthritis Proc. NATL. Acd. Sci. USA. 85(12), 4553-4556.

Levitt, B. and Westfall, D.P. (1982). Factor influencing the release of purines and norepinephrine. Blood Vessel. 19, 30-40.

Lewis, T., and Grant, R.T. (1924): Vascular reactions of the injury: The liberation of histamine like substance in injured skin; The underlying cause of factitious urticaria and of wheals produced by burning; and observations upon the nervous control of certain skin reactions, Heart. 11,209.

Li, G. ,Bronk, J.T. ,Kelly, P.J. (1989). Canine bone blood flow estimated with microspheres. J. Orthop. Res. 7(1), 61-67.

Liew, M. ,and Dick,W.C. (1981). The anatomy and physiology of blood flow in diarthrodial joint. Clin. Rheum. Dis. 7, 131-148.

Loewi, O. (1921). Uber humorale uber tragbarkeit der herznervenwirkung, I. milleilung. Pfluger's Archives. 189, 239-242.

Lucht, U. ,Bunger,C. ,Krebs,B. ,Hjermind,J. Bulow,J. (1983). Blood flow in the juvenile hip in relation to changes of the intraarticular pressure. Acta. Orthrop. Scand. 54, 182-187.

Lundberg J.M., Anggard A., Theodorsson-Noheim E., & Pernow J. (1984). Guanethidine-sensitive release of neuropeptide Y-like immunoreactivity in the cat spleen by sympathetic nerve stimulation. Neuroscience Letters 52: 175-180.

Lunde, P.K.M. ,and Michelsen,K.(1970). Determination of cortical blood flow in rabbit femur by radioactive microspheres. Acta. Physiol. Scand. 80, 39-44.

Lynn, B. Shakhanbeh, J. (1989). Axon reflex flare in anaesthetized rabbit skin. J. Physiol. 414, 37p.

Maclennan, S.J. ,McGrath, J.C. ,Whittle, M.J. (1988). Inhibition of the oxygen-induced contraction of the isolated human umbelical artery by indomethacin, flurbiprofen, asperin and drugs modifying Ca^{2+} disposition. Prostaglandins. 36(5), 711-729.

May, W.P. (1904). The innervation of the sphincters and musculature of the stomach. J. Physiol. (London) 31, 260-271.

McDonald, J.K. (1988). NPY and related substances. Critical review in neurobiology. vol 4, Issue 1, 97-135.

McGrath, J.C. (1981). Vascular adrenergic receptors. Vasodilation, Eds. Vanhoutte, P.M. and Leusen, I. raven press, New York. 97-106.

McGrath, J.C. (1982). Evidence for more than one type of alpha-adrenoceptor. Biochemical Pharmacology. 31, 467-484.

McGrath, J.C. (1983). The variety of vascular alpha-adrenoceptors, TIPS. 4,(1), 14-18.

McSwiney, B.A. and Robson, J.M. (1929). The response of smooth muscle to stimulation of the vagus nerve. J. Physiol. 68, 124-131.

Minneman, P.K. (1988). α_2-adrenergic receptor subtypes, Inositol Phosphates, and sources of cell Ca^{2+}. Pharmacol. Rev. 40(2), 87-119.

Mitchell, G.A.G. (1953). Anatomy of the autonomic nervous system. E.S. Livingstone, Ltd., Edinburgh.

Morris, M.A. ,Kelly, P.J. (1980). Use of tracer microspheres to measure bone blood flow in conscious dogs. Calcif. Tissue Int. 32, 69-76.

Moulds, R.F.W. and Jauernig, R.A. (1977). Mechanism of prazosin. Lancet. 1, 200-201.

Moylan, R.D. & Westfall, T.C. (1979): Effect of adenosine on adrenergic neurotransmission in the superfused rat portal vein. Blood Vessels 16: 306-310.

Muramatsu I., Fuginara M., Muira A. & Sakakibara Y. (1981). Possible involvement of adenine nucleotides in sympathetic neuroeffector mechanisms of dog basilar artery. Journal of Pharmacology and Experimental Therapeutics 216: 401-409.

Nakanishi, H. & Takeda, H. (1972): The possibility that adenosine- triphosphate is an excitatory transmitter in guinea-pig seminal vesicle. Japanese Journal of Pharmacology 22: 269-270.

Nelemans, F.A. (1951). Liberation of sympathin and acetylcholine by faradic stimulation of the frog's heart. Acta. Physiol. Pharmacol. Neer. 2, 51-62.

Nield, T.O. & Zelcer, E. (1982): Noradrenergic neuromuscular transmission with special reference to arterial smooth muscle. Progress in Neurobiology 19: 141-158.

Nielsen, K.C. ,and Owman, Ch. (1968). difference in cardiac adrenergic innervation between hibernators and nonhibernating mammals. Acta. Physiol. Scand. 316(suppl.), 1-30.

Nillson, G.E., Tenland, R. ,and Oberg, P.A. (1980). A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. IEEE Trans. Biomed. Eng. BME. 27, 12-19.

Notzli, H.P. ,Swiontkowski, M.F. ,Thaxter, S.T. ,Carpenter, G.K.II, Wyat, R. (1989). Laser Doppler flowmetry for bone blood flow measurements Helium-Neon laser light attenuation and depth of perfusion assessment. J. Orthop. Res. 7(3), 413-424.

Oliver, G. and Schafer, E.A. (1895). The physiological effects of extracts from suprarenal capsules. J.Physiol. 18, 230-237.

Paton, W.D.M. and Vane, J.R. (1963). An analysis of the responses of the isolated stomach to electrical stimulation and to drugs. J. Physiol. 165, 10-46.

Pearson, C.M.,and Wood, F.D. (1959). Studies of polyarthritis and lesion induced in rat by injection of micobacterial adjuvant. Arthritis Rheum. 2, 440-459.

Phillips, D.S. (1978): Basic statistics for health science students. W.H. Freedman and Company, San Fransisco.

Potter, D.D., Furshpan, E.J. and Landis, S.C. (1983). Transmitter status in cultured rat sympathetic neurons : plasticity and multiple function. Fed. Proc. 42, 1626-1632.

Powell, J.R. and Brody, M.J. (1976). Participation of H_1 and H_2 histamine receptors in physiological vasodilator

responses.Am, J. Physiol 231, 1002.

Powell, C.E. ,and Stater, I.H. (1958). Blocking of inhibitory adrenergic receptors by a dichloro-analog of isoproterenol. J. of Pharmacology and Experimental Therapeutics. 122, 480-488.

Raab, W.H.M. ,Magerl, W. ,and Muller, H. (1988). Changes in dental blood flow following electrical tooth pulp stimulation-influences of capsaicin and guanethidine. Agents and Action. 25, 237-239.

Riva, C. ,Ross, B. ,and Benedict, G.B. (1972). Laser Doppler measurements of blood flow in capillary tubes and retinal arteries. Inves. Ophthalmol. 11, 936-944.

Rudolph, A.M., and Heymann, M.A. (1967). The circulation of the fetus in utero: methods of studying distribution of blood flow, cardiac output, and organ blood flow. Circ. Res. 21, 163-184.

Sammuel, E.P. (1952). The autonomic and somatic innervation of the articular capsule. Anat. Rec. 113, 53-70.

Schaible H-G & Schmidt R.F.(1988) Direct observation of the sensitization of articular afferents during an experimental arthritis. In: Proceedings of the Vth World Congress on Pain eds. DubnerR., Gebhart G.F. & Bond M.R.

Elsevier Science Publishers BV (Biomedical Division).

Schlesinger, M.J. (1957). New radiopaque mass for vascular injection. Lab Investigator. vol 6, (1). 1-11.

Schon, F. ,Allen, J.M. ,Yeats, J.C. ,Allen,Y.S. ,Ballesta, J. , Polak, J. M. , Kelly, J.S. ,and Bloom, S.R. (1985). Neuropeptide Y innervation of the rodent pineal gland and cerebral blood vessels. Neurosci. Lett. 57, 65.

Semba, T., Fujii, K., and Kimura, N. (1964). The vagal inhibitory responses of the stomach to stimulation of the dog's medulla oblongata. Jap. J. Physiol. 14, 319-327.

Shepherd, A.P. ,and Riedel, G.L. (1982). Continuous measurement of intestinal mucosal blood flow by laser-Doppler velocimetry. Am. J. Physiol. 242, G668-G672.

Shim, S.S. (1968). Physiology of blood circulation of bone. J. Bone and Joint Surgery. 50A, 812-824.

Simkin, P.A. ,Huang,A. ,and Benedict, R.S. (1986). Exercise induces bidirectional changes in blood flow to canine articular tissues. Scand. J. Rheum. (Suppl.) 60, A113.

Skoglund, S. (1956). Anatomical and physiological studies of knee joint innervation in the cat. Acta. Physiol. Scand. 36(suppl. 124), 1-101.

Sneddon, P. & Burnstock, G. (1984a). Inhibition of excitatory junction potentials in guina-pig vas deferens by α, ß -methylene ATP: further evidence for ATP and noradrenaline as cotransmitters. Eur. J. Pharmacol. 100: 85-90.

Sneddon, P., and Burnstock, G. (1984b): ATP as a co-transmitter in rat tail artery. European Journal of Pharmacology 106: 149-152.

Sneddon, P., Westfall, D.P. (1984). Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in the guinea-pig vas deferens, J.Physiol (London). 347, 561-580.

Starke, K. ,Borowiski, E. and Endo,T. (1975a). Preferential blockade of presynaptic alpha-adrenoceptors by Yohimbine. Europ. J. of Pharmacol. 34, 385-388.

Starke, K. ,Endo,T. and Taube, H.D. (1975b). Relative pre and post synaptic potencies of alpha-adrenoceptor agonists in the rabbit pulmonary artery. Naunyn-Schmiedeberg's Archives of Pharmacol. 291, 55-78.

Starke, K. ,Endo,T. and Taube, H.D. (1975c). Pre- and post synaptic components in effect of drugs with alpha-adrenoceptor affinity. Nature. (London) 254, 440-441.

Stern, M.D. (1975). In-vivo evaluation of microcirculation by coherent light scathering. Nature. 254, 56-58.

Starke, K., and Montel, H. (1973). Alpha-receptor mediated modulation of transmitter release from central noradrenergic neurons. Naunyn-Schmiedebergs Arch. Pharmacol. 279,53.

Stren, M. D. ,Bowen, P.D. ,Parma, R. ,Osgood, R.W. ,Bowman, R.L. ,and Stein, J.H. (1979). measurment of the renal cortical and medullary blood flow by laser-Doppler spectroscopy in the rat. Am. J. Physiol. 236, F80-F87.

Stern, M.D. ,Lappe, D.L. , Bowen, P.D. , Chimosky, J.E. ,Holloway,Jr. G.A. , Keiser, H.R. , and Bowman, R.L. (1977).Continuous measurement of tissue blood flow by Laser-Doppler spectroscopy. Am. J. Physiol. 232, H441-H448.

Stevens M.J. & Moulds R.F.W. (1985). Neuronally released norepinephrine does not preferentially activate postjunctional ı-adrenoceptors in human blood vessels in vitro. Circulation Research 57: 399-405.

Su, C. (1975): Neurogenic release of purine compounds in blood-vessels. Journal of Pharmacology and Experimental Therapeutics 195: 159-166.

Su, C. (1977): Purinergic inhibition of adrenergic transmission in rabbit blood vessels. Journal of Pharmacology and Experimental Therapeutics 204: 351-361.

Su, C. (1978): Modes of vasoconstriction and vasodilator neurotransmission. Blood Vessels 15: 183-189.

Su, C. (1981): Purinergic receptors in blood vessels. In purinergic receptors (Receptor and Recognition, Series B. vol 12). ed. Burnstock, G. pp. 1-45. London: Chapman and Hall.

Su, C. (1983). Purinergic neurotransmission and neuromodulation . Ann. Rev. Pharmacol. Toxicol. 23, 397-411.

Su, C., Bevan, J.A., and Burnstock, G. (1971). [^{3}H]-Adenosine triphosphate: release during stimulation of enteric nerves. Science. 173, 337-339.

Swiontkowski, M.F. ,Tepic, S. Ganz, R. Perren, S.M. (1986a). Laser Doppler flowmetry for measurement of femoral head blood flow: Experimental investigation and clinical application. Helv. Chir. Acta. 53, 55-59.

Swiontkowski, M.F. ,Tepic, S., Perren, S.M. ,Moor, R. ,Ganz, R. ,Rahn, B.A.(1986b). Laser Doppler flowmetry for bone blood flow measurement correlation with microsphere estimates and evaluation of the effect of intracapsular pressure on femoral head blood flow. J. Orthop. Res. 4(3), 362-371.

Swiontkowski, M.F. , Schlehr, F. ,Collins, J.C. ,Sanders, R. ,Pou, A. (1988). Camparison of two laser Doppler flowmetry systems for bone blood flow analysis. Calcif. Tissue Int. 43(2), 103-107.

Timmermans, P.B. M.W.M. and Van Zwieten, P.A.(1981). The postsynaptic α_2-adrenoceptor. J. of Autonomic Pharmacol. 1, (2), 171-183.

Timmermans, P.B. M.W.M. and Van Zwieten, P.A.(1982). α_2-adrenoceptors: classification, location, mechanisms and targets for drugs. J. of Medicinal Chemistry. 25, 1389-1401.

Tothill, P.(1984). Bone blood flow measurement. J. Biomed. Eng. 6, 251-256.

Uvnas, B. (1960). Central cardiovascular control. In handbook of Physiology, section 1: Neurophysiology, vol. II, ed. by J.Field. pp.1131-1162. American Physiological Society, Washington, D.C.

Vanhoutte, P.M. & Rimele, T.J. (1983): Role of the endothelium in the control of vascular smooth muscle function. J. Physiol. (Paris) 78: 681-686.

Vizi E.S.(1979). Presynaptic modulation of neurochemical transmission. Prog. Neurobiol. 12: 181-290.

Wakade, A.R. & Wakade, J.D. (1978): Inhibition of norepinephrine release by adenosine. Journal of Physiology 282: 35-49.

Wallis, W.J. ,Simkin,P.A. ,Nelp,W.B. ,and Foster, D.M. (1985). Intra-articular volume and clearance in human synovial effusion. Arthritis Rheum. 28, 441-449.

Weinner, N. and Taylor, P. (1985). Drugs acting at synaptic and neuroeffector junctional sites. In the Pharmacological basis of therapeutics, chap. 4, ed. Goodman, A.G. Gillmann, L.S. ,Rall, T.W. and Murad, F. pp.66-99. New York: Macmillan Publishing Company.

Weinner, N. and Taylor, P. (1985). Alpha-adrenergic Blockers. In the Pharmacological basis of therapeutics, chap. 9, ed. Goodman, A.G. Gillmann, L.S. ,Rall, T.W. and Murad, F. pp.181-214. New York: Macmillan Publishing Company.

Weitzell, R. ,Toshiyaki, T. and Starke, K. (1979). Pre- and post synaptic effect of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. Nauyn-Schiedeberg's Archives of Pharmacology. 308, 127-136.

Westfall, T.C.(1977): Local regulation of adrenergic neurotransmission. Physiological Reviews 57: 659-728.

Westfall, T.C., Stitzel, R.E., and Rowe, J.N. (1978): The postjunctional effects and neuronal release of purine compounds in the guinea pig vas deferens. European Journal of Pharmacology 50: 27-38.

Zimmerman, B.G. (1968). Camparison of sympathetic vasodilator innervation of hindlimb of the dog and cat. Am. J. Physiol. 214, 62-66.

Zimmerman, H., Dowdall, M.J., and Lane,D.A. (1979). Purine salvage at the cholinergic nerve endings of the torpedo electric organ-central role of adenosine. Neuroscience. 4, 979-993.

****

****

****

****

***

*