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博士論文

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Abstract

To examine the direct effects of norepinephrine (NE) and serotonin (5-HT)on the contractility of arterioles in the gray matter of the rat cerebrum, I microperfused arterioles in vitro and observed the changes in luminal diameter under the stop-flow condition with constant intraluminal pressure.

The lumen of an arteriole perfused in vitro was kept constant by minimizing the luminal flow after clamping the distal end and by keeping the intraluminal hydrostatic pressure at 30mmHg.

In Hepes-buffered saline, the average diameter of the lumen of arteioles was 39.9±3.7 μm(n=7). NE at 10^{-7} M in the extraluminal solution caused contraction of arterioles by 21.1±2.0% in diameter(n=7). While the contractile response to 10^{-6} M NE was not attenuated by 10^{-8} M prazosin, an alpha-1 adrenagic antagonist, yohinbin, an alpha-2 blocker, significantly reduced the contractility of the arterioles in response to NE. The contractile effect of NE was dose-dependent between the 10^{-7} and 10^{-5}M in seven arterioles.

10^{-6} M NE applied to the lumen also caused contraction of arterioles by 14.8 ± 3.1% in diameter (n=5)in 5min.

The effect of serotonin on the contractility of arterioles was also observed. Serotonin added to the extraluminal solution at 10^{-10} M caused contraction of arterioles by 10.9±1.5% in diameter(n=8)in 5 min. Serotonin in the extraluminal solution caused contraction of arterioles in a dose dependent manner between 10^{-10} and 10^{-6}M. The contractile effect of serotonin at 10^{-6} M was strongly reduced by 10^{-6}M ketanserin, a 5HT-2 receptor antagonist.

While norepinephrine in the lumen caused contactionof arterioles, serotonin applied to the lumen had no effect at all.

These results strongly suggest that serotonin plays a significant role in arteriolar contractility only from the CSF side, while norepinephrine is an important regulator of arteriolar contractility from both the CSF and blood circulation sides.

introduction

Among various types of vasoactive substances, norepinephrine(NE) and serotonin (5-hydroxytryptamine,5HT)are the most well-known and well investigated compounds. NE has thus far been reported to cause contraction of arteries and arterioles in various tissues.

Cerebral arteries are richly innervated by sympatheic nerves, the arteries being relatively unresponsive to norepinephrine, compared with the response of vessels in other organs(Bevan et al.1987). The vasoactive effet of NE on the cerebrovascular system in vitro was first discribed by Bohr et al(Bohr et al., 1961),who reported that there are marked differences in the sensitivity of isolated dog cerebral and peripheral arteries to vasoactive substances including NE. After this report, there were many experiments on the precise location and mechanism of NE's cerebrovascular reactivity.

It then became obvious that the concept that norepinephrine causes constriction of vessels strongly dose not always apply to cerebral vessels. While contractile responses to NE via alpha receptors were observed in isolated cerebral arteries of guinea pig(Chang et al., 1988), monkey(Chang et al. , 1987., Toda, 1991), and beagle(Toda et al., 1986), relaxant responses were also observed in isolated bovine caudal arteries (Ayajiki and Toda, 1992), and porcine cerebral arteries(Winquest et al., 1982). Further, more Hempelmann and Zieglar(1993) reported a relaxant response to NE of isolated rat cerebra arteries, which depended upon the precence of an intact endothelium. Kitazono(1993) showed that the topical appllication of NE increased of the diameter of the basilar artery in a cranial window preparation. They suggested that the dilatation was due to activation of the beta-1 receptors and that it was not mediated by endothelium-derived relaxing factor(EDRF). On the other hand, Chang, Hardebo et al.(1988)reported that NE had no effect on isolated rat basilar arteries from rats. These controversial results regarding the sensitivity of the cerebrovascular system to NE lead me to the idea that cerebrovascular system is heterogenous in its response to NE.

Another well-known classical vasoactive substance, the physiolaogical role of

which remains uclear, is 5-HT. In 1973, Toda and Fujita found, in isolated spiral atrips of cerebral arteries from dog and man, that 5-HT caused martkedly contraction of arteries, the magnitude of which was greater than in the case of NE(Toda and Fujita, 1973). Similar results for isolated artery preparations were reported in rat(Chang et al.,1988., Foster and Whalley, 1982), cat(Hardebo et al., 1978), guinea pig(Chang et al., 1988), monkey(Chang et al., 1987, Toda, 1991), and man(Hardebo et al.,1978). Therefore , the effects of 5-HT on isolated cerebral arteries thus far reported are in good agreement with each other in that 5-HT is a potent contractile agent in different species and brain regions.

Grome and Harper(1983), however, showed that the intracarotid infusion of 5-HT into rats did not change the cerebral blood flow except for in the caudal nucleus. Although there are several hypotheses, the most possible explanation for the discrepancy between in vivo and in vitro studies is that 5-HT exerts its vasoconstrictive effect only from the extraluminal side. This idea is supported by some studies demonstrating that intravenously administered 5-HT receptor agonists exhibit poor ability to penetrate the cerebrovascular intimal layer(Conner et al.,1992,. Ferrari, 1993).

It is therefore important to examine the vasoreactivity of the cerebrovascular system to NE and 5-HT with an in vitro preparation which allows the detection of the intraluminal and extraluminal effects of these substnaces separately. Considering the lack of a report demonstrating directly the laterality of the vasoactivity of NE and 5HT in cerebral arterioles, I conducted a series of experiments on the direct effects of intraluminal and extraluminal NE and 5-HT on an in vitro microcanulated preparation. I strongly suggest from my results that NE is a potent vasoconstrictive substance for cerebral arterioles from both the systemic blood flow and cerebrospinal fluid (CSF)sides, whereas 5-HT exerts its vasoconstrictive effect on cerebral arterioles only from the CSF side.

materials and methods

The techniques used for the dissection and canulation of intracerebral arterioles were those of Dacey and Duling(Dacey and Duling, 1982) with the modification of Kondo and Frömter's in vitro renal tubule microperfusion technique(Kondo and Frömster,1987).

iolation of arterioles

Male Wister rats weighing 300-400g were anesthetized with pentobarbital sodium (Nembutal, Abbott; 50mg/kg i.p.)and then decapitatd with a small-animal guillotine. The top of the calvarium was removed with a drill, and then the temporal and parietal bones were fractured laterally with opthalmological surgical scissors. The dura was then removed and the brain was rapidly immersd in a dissection chamber at 4 ℃ containing a Hepes-buffered solution, the composition of which was as follows, in mM; .135NaCl,3KCl,2KH_2 PO_4, 1.5$CaCl_2$, 1.0$MgCl_2$, 10Hepes, 5.5glucose, 5l-alanine titrated to PH 7.4 with NaOH. Under a stereomicroscope(SZ40R., Olympus, Tokyo, Japan),a piese of cerbral cortex approximately 2 mm thick was removed from the lateral cerebral hemispheric surface. The pia mater and the attached penetrating arterioles were separated from the parenchyma with fine forceps, and an unbranched segment of an intracerebral arteriole of approximately 0.7 to 1.0mm in length and 20 to 40 μm indiameter was cut with a 29G needle .

The arterioles were then transferred to a temprature-controlled chamber, containing the above Hepes-buffered solution, which was mounted on the stage of an inverted microsope(IMT-2., Olympus,Tokyo).

in vitro cannulation of arterioles

The isoalated arterioles were cannulated using a system of concentric glass pipetts mounted on micromanipulators(in vitro microperfusion manipulators,. Narishige, Tokyo)attached to the microscope stage. By applying gentle suction, one end of an arteriole was drawn into a holding pipette through the constriction. A perfusion pipette was then inserted into the lumen of the arteriole. The inner diameters of the holding and perfusion pipettes were 30 to 36 and 3 to 5 μm, respectively. To stop the luminal flow completely, the other end was wedged with another holding pipette, the inner diameter of which was 30-40μm. After canulation, the intraluminal pressure was constantly maintained at 30 mm Hg by means of hydrostatic pressure . The Hepes-buffered solution described above was used to perfuse both the luminal and extraluminal sides of arterioles.

To ensure the stop-flow of the lumen, arterioles with holes or branches were discarded. After an equilibration period of approximatly 30 minutes, spontaneous tone developed. Images of the arterioles were monitored at the magnification of 200x with a video camera(C-2400-08,. Hamamatsu Photonics,Hamamatsu), and the luminal diameters of three random points were measured with an image-analysis system(ARGUS-10,. Hamamatsu Photonics,Hamamatsu).

The viability and biological responsiveness of the arterioles were then asessed by applying a high K^+ solution instead of 124 mM NaCl with the same molar concentration of KCl to the extraluminal side. Arterioles exhibiting a weak response to high K^+, namely less than 20% in diameter contraction, were excluded from the data collection.

Chemicals

Serotonin(5-Hydroxytryptamine,5-HT), L-norepinephrine hydrochloride, (norepinephrine, NE), prazosin hydrochloride and yohinbin hydrochloride were purchased from Sigma(Mo, USA). Ketanserin tartrate and methiothepin mesylate were from RBI(MA, USA). All other chemicals were of reagent grade and were purchased from Wako Pure Chemicals(Osaka, Japan).

statistics

All values were expressed as means ±SE. All statistical analyses were performed using percent changes from control values. A paired Student's t-test was used to compare mean values btween two groups. A value of $P<0.05$ was considered significant.

Results

basal contractile response assesed as the high K^+-induced contractility of arterioles

After a 30 minute pre-equilibration period, the average luminal diameter of the arterioles was 39.9±3.7 μm(n=7).

It is well known that the depolarization of arteriolar smooth muscle cells by increasing extracellular K^+induceds an increase in intracellular Ca^{++}, followed by their contraction. To confirm the viability and biological contractility of the arterioles, the extraluminal K^+concentration was increased to 124mM by adding the same molar concentration of Na^+, and then the percent contractility of the arteriolar luminal diameter in 5 min was determined. The change in the luminal diameter in response to the extraluminal high K^+solution is depicted in Fig.1. Because the contractility response to high K^+ is an essential phenomenon which proves that cells retain their physiological intracellular environment, the group of arterioles exhibiting low contractility was regarded as an inappropriate preparation and thus discarded. To eliminate this group used in the present study, all arterioles exhibiting less than 20% contractility were discarded.

contractile response to extraluminal NE

The vessel diameter exhibited maximal steady-satate contraction at 5 min after the administration of NE.

a representative time course of the contractile response of arterioles to 10^{-6}M NE is shown in Fig.2. As shown in Fig.3, 10^{-7}, 10^{-6}, 10^{-5}M NE decreased the arteriolar diameter by 21.1±2.0, 31.1±1.9, and 37.6±1.1%(each n=7), respectively.

To determine whether or not alpha-1and alpha-2 adrenergic receptors were involved in the contractile responses to NE, arterioles were first incubared with either 10^{-8}M prazosin a selective alpha-1 antagonist or 10^{-6}M yohinbin,an alpha-2 antagonist, for 5 min in the extraluminal solution, and then the contractile responses to 10^{-6}M NE were observed in the presence of these antagonists.

Neither prazosin noryoninbin itself affected the luminal diameter of the arterioles. In the presence of prazosin, 10^{-6}M NE caused contraction of the arterioles by 21.1±3.9%, which was the same as the response in the absence of prazosin(28.3±

2.7%, n=5, n.s. in paired t-test)(Fig.3). In the presence of yohinbin in the extraluminal solution, the contractile response decreased to 4.9±1.7%(n=5,$p<0.05$ versus control in paired t-test)(Fig.4.).

These results clearly show that NE caused contraction of the arterioles via alpha-2 adrenergic receptors, which may be located on either the plasma membrane of the smooth muscle cells or the extraluminal surface of the endothelium.

Contractile response to luminl NE

The intraluminal application of 10^{-6}M NE also decreased the arteriolar diameter by 14.8±3.1%(n=5), this magnitude being less than that of the extraluminal response ($p<0.05$, Fig.5.).

Contractile response to extraluminal 5-HT

After a 30 minute preincubation period, the luminal diameter of the arterioles was 35±5μm(n=8). A representative time course of the responses of arterioles to 5-HT is shown in Fig.6. 10^{-10}, 10^{-8}, and 10^{-6}M 5-HT decreased the arteriolar diameter by10.9±1.5, 16.2±8.4, and 18.5±1.0%(n=7), respectively(Fig.7.).

To determine whether or not 5-HT_1 and 5-HT_2 receptors are involved in the contractile response to 5-HT, the arterioles were first incubated with either 10^{-6}M methiothepine, a 5-HT_1 antagonist, or 10^{-6}M ketanserin a 5-HT_2 antagonist, for 5 min in the extraluminal solution, and then the contractile response to 5-HT were observed in the presence of these antagonists.

Neither methiothepine nor ketanserin itself affected the luminal diameters of the arterioles. In the presence of methiothepine, 10^{-6}M 5-HT caused contraction of the arterioles by 9.7±2.5%, which was the same as the response in the absence of methiothepine(13.9±1.4%,n=5, n.s. in paired t-test),as shown in Fig.8. In the presence of ketanserin in the extraluminal solution, the contractile response decreased to 2.9±0.9%(n=5. $p<0.05$ versus control in paired t-test), as shown in Fig.8.

These results clearly show that 5-HT caused contraction of the arterioles via 5-HT_2 receptors which may be located on either the plasma membrane of the smooth muscle cells or the extraluminal surface of the endothelium.

Contractile response to luminal 5-HT

The intraluminal application of 10^{-6}M 5-HT decreased the luminal diameter of arteriole by 0.1±0.1%(n=6)in 5 min, which was not a statistically significant change (Fig.9.).

It is clear from these results that 5-HT has no receptor on the luminal membrane of the arteriolar endothelium, and thus it modulates contractility only from the cerebral blood circulation side.

Discussion

In the present study, I demonstrated the precise laterality of the effects of classical vasoactive substances, NE and 5-HT. My data indicate differences in the physiological roles of these two substances in light of the locations of the receptors.

My data alos show that intracerebral arterioles , which serve the terminal resistance vessels contributing to blood pressure hemostasis as the final regulator in the cerebrum, are induced to contract by NE from both sides of them. It is well established that NE is impermeable to cerebral blood vessels under physiological conditions(Hardebo et al., 1977,.Weil-Malherbe et al.,1961). Therefore, I strongly suggest that intracerebral arterioles are regulated by NE from both the blood circulation and CSF sides.

As for the effects of NE on the cerebrovascular system, in vivo experiments in man (Greenfield and Tindal, 1968)and dog(Ekstroem-Jodal and Haggendal, 1974)demonstrated that the intravenous infusion of NE induced a reduction in cerebral blood flow(CBF) via alpha-adrenergic receptors. On the other hand, the same experimental protocol in rat showed that NE increased CBF via beta-adrenergic receptors(Berntman et al., 1978). This type of experiment dose not always allow a clear conclusion as to the contractile response of cerebral arteries to NE, because CBF is determined not only by vascular resistance but also by regional blood pressure. Although direct analysis of the effect of NE was performed on in vitro isolated vessels, including basilar, pial and carotid arteries, it must be noted that all in vitro helical strips and ring preparations examined exhibited the mixed-up phenomena caused by the stimulation of both intraluminal and extraluminal adrenergic receptors.

It is also important that the majority of studies have been on the arterial contractility induced by NE in relatively large arteries, including basilar, pial and carotid arteries. As for intracerebral arterioles, the first experiment involving the in vitro microperfusion technique was performed by Dacey and Duling (Dacey and Duling, 1982,. Dacey and Duling , 1894,. Duling et al., 1981). They reported that a physiological dose of NE caused contraction of the arterioles from the extraluminal side via alpha-adrenergic receptors(Dacey and Duling, 1984). Their data are in good accordance with mine in that cerebrovasular NE caused contraction of the arterioles at a physiological concentration. My data further demonstrated that the contractile effet of NE

is mediated by alpha-adrenergic receptors.

Thus far, there has been no study on the effect of intraluminal NE on the vascular contractility of intracerebral arterioles as to NE. My data demonstrate clearly that NE in the cerebrovascular system is also a potent vasoconstrictive factor for the intracerebral arterioles and regulates the cerebrovascular circulation. At present, no information is aveilable on how intraluminal NE causes contraction of smooth muscle cells, which have no direct contact with an intraluminal solution. Another series of experiments on the mechanism of signal transduction of the NE effect is required.

In the present study, I did not examine whether or not the beta-adrenergic receptors were also involved in the vasoconstractile response of intracerebral arterioles, as was reported in previous studies(Berntman et al., 1978,. Kitazono et al., 1993). In the case of my data, inhibition of alpha-2 adrenergic receptors by yohinbin did not change the effet of NE on arterioles from vasoconstriction to dilatation. Although this result implies the absence of beta-adrenergic receptors in intracerebral arterioles, more direct examination of the effects of NE are required to elucidate whether or not functional beta receptors are present in intracerebral arterioles.

5-HT is another classical vsoconstrictive substance, the role of which in regulation of the cerebrovascular system remains unclear. In migrane, antagonists to 5-HT receptors are often applied for therapeutic use, because one of the causes of migrane is thougt to be a disturbance of a 5-HT related vasoconstrictive system in the brain(Ferrari, 1993). In anesthetized baboons(Harper and Mackenzie, 1977)and rats(Grome and Harper, 1983), direct intravenous infusion of 5-HT in vivo had no effect on cerebrovascular blood flow except for in the caudal nucleus. My data are in good agreement with those of the previous in vivo studies demonstrating the absence of an intraluminal receptor for 5-HT in whole brain vessels. It is well established that the BBB is impermeable to 5-HT. Several in vitro studies demonstrated the occurrence of a vasoconstrictive action of 5-HT in arteries and arterioles, including basilar, carotid arteries and intracerebral arterioles(Chang et al., 1988,. Mylecharance, 1990,. Saxena and Villalon, 1990). The preparations used were in vitro herical strips or simply isolated vessels. Therefore, these data do not necessarily indicate the presence of intraluminal vasoconstrictive receptors for 5-HT. Rather, it seems more reasonable that all the observed vasoconstrictive effects of 5-HT are exerted from the extraluminal

side. There has only been one preliminary report demonstrating the contractile effect of 5-HT on in vitro microperfused intracerebral arterioles from the lumen(Ogura et al., 1991). I do not know why the vasoconstrictive effect of 5-HT from the lumen was observed in their preparation. To clarify the discrepancy between their data and mine, I must wait until their data are published in a precise form.

Figure legends

Fig.1. Contractile response of intracortical arterioles to an extracellular high k^+solution.

To examine the viability of arterioles , contractility was examined by applying a high k^+solution to the extraluminal side, and then changes in the inner diameter of the arterioles were observed. In this figure, the arterioles which contracted by less than 20% in diameter were ignored. In controls, the inner diameter of arterioles was 52.0± 1.9 μm. After 5 minutes exposure of the arterioles to the high k^+solution containing 124mMk^+, the inner diameter was reduced to 31.2 ±1.7 μm(n=5,$p<0.05$), corresponding to 42.4±3.1% contraction.

Fig.2. Representative time course of contraction induced by extraluminal NE.

Representative time course of contractile responses of arterioles to 10^{-6} M NE in the extraluminal solution is shown. Contractile responses, as inner diameters of the arterioles, were plotted against time in minutes for two arterioles. As depicted, the maximal contractile responses were observed at approximately 5 to 10 minutes after the administraion of NE.

Fig.3. Dose-dependent contractility of arterioles in response to extraluminal NE.

The effect of NE at various concentrations applied from the extraluminal side was examined. NE, from 10^{-7} to 10^{-5} M, was applied to extraluminal side, and then the contractile responses, as percent changes of the inner diameter, were observed. NE at 10^{-7}, 10^{-6}, and 10^{-5}M caused contration of the arterioles in 5 min by 21.1±2.0, 31.6± 1.8., and 37.6±1.1%(n=7), respectively.

Fig.4. Effects of prazosin and yohinbin on NE-induced contracion of arterioles.

To determine whether or not NE-induced contraction of arterioles was mediated by alpha-1 or alpha-2 adrenergic receptors, the contractile responses to 10^{-6}M NE were observed after preincubating the arterioles with either 10^{-6}M yohinbin, an alpha-2 antagonist, or 10^{-8}M prazosin, an alpha-1 antagonist. These doses of the antagonists are known to inhibit the specific receptors maximally. Each antagonist by itself had no contractile or dilating effect on arterioles during preincubation for more than 5 min. The

antagonists were applied to the same arteioles, but in random order.In controls, 10^{-6} M NE caused contraction of the arterioles by 28.3±2.7%(n=5). Preincubation of the arterioles with 10^{-6} M yohinbin reduced the contractility to 4.9±1.7% in 5 min(n=5, $p<0.05$ versus control), whereas NE, with preincubation of 10^{-8} M prazosin for 5 min, still caused contraction of the arterioles by 21.1±3.9%(n=5, n.s. versus control).

Fig.5. contractile effect of NE applied from the lumen.

To determine whether or not NE was capable of causing contraction of arterioles from the intraluminal side, 10^{-6} M NE was applied to the lumen by simple diffusion from the tip of a perfusion pipette without perfusing the lumen. To accomplish the diffusion, only the inside of the perfusion pipette was exchanged to the solution containing to 10^{-6} M NE, without any change in the hydrostatic pressure, carefully. As is shown in the figure, NE applied to the intraluminal side caused contraction of the arterioles by 12.4± 2.4%(n=5)in 5 min.

fig.6. Representative time course of contraction induced by extraluminal 5-HT.

Representative time course of contractile responses of arterioles to 10^{-6} M 5-HT in the extraluminal solution is shown. Contractile responses , as inner diameters of the arterioles, were plotted against time in minutes for two arterioles. As depicted, the maximal contractile responses were observed at approximately 5 to 10 minutes after the administration of 5-HT.

Fig.7. Dose-dependent contractility of arterioles in response to extraluminal 5-HT.

The effect of 5-HT at various concentrations applied from the extraluminal side was examined. 5-HT, from 10^{-10} to 10^{-6} M, was applied to the extraluminal side, and then the contractile responses , as percent changes of the inner diameter, were observd. 5-HT at 10^{-10}, 10^{-8}, 10^{-6} M caused contraction of the arterioles in 5 min by 10.9±1.5, 16.2±8.4, and 18.5±1.0%(n=7), respectively.

Fig.8. Effects of methiothepine and ketanserin on 5-HT induced contraction of arterioles

To determine whether or not 5-HT induced contraction of arterioles was mediated by 5-HT_1 or 5-HT_2 receptors, the contractile responses to 10^{-6} M 5-HT were

observed after preincubating the arterioles with either 10^{-6} M methiothepine , a 5-HT_2 antagonist, or 10^{-6} M ketanserin, a 5-HT_1 antgonist. These doses of the antagonists are known to inhibit the specific receptors maximally. Each antagonist by itself had no contractile or dilating effect on the arterioles during preincubation for more than 5 min. The antagonists were applied to the same arterioles, but in random order. In controls, 10^{-6} M 5-HT caused contraction of the arterioles by 13.9±1.4%(n=5). Preincubation of the arterioles with 10^{-6} M ketanserin reduced the contractility to 2.0±0.9% in 5 min (n=5, $p<0.05$ versus control), whereas 5-HT, with preincubation of 10^{-6} M methiothepine for 5 min, still caused contraction of the arterioles by 9.7±2.5%(n=5, n.s. versus control).

Fig.9. Contractile effect of 5-HT applied from the lumen.

To determine whether or not 5-HT was also capable of causing contraction of arterioles from the intraluminal side, 10^{-6} M 5-HT was applied to the lumen by simple diffusion from the tip of a perfusion pipette without perfusing the lumen. As shown in the figure, 5-HT applied to the intraluminal side caused contraction of the arterioles by only 0.1±0.1%(n=6, n.s. versus zero)in 5 min.

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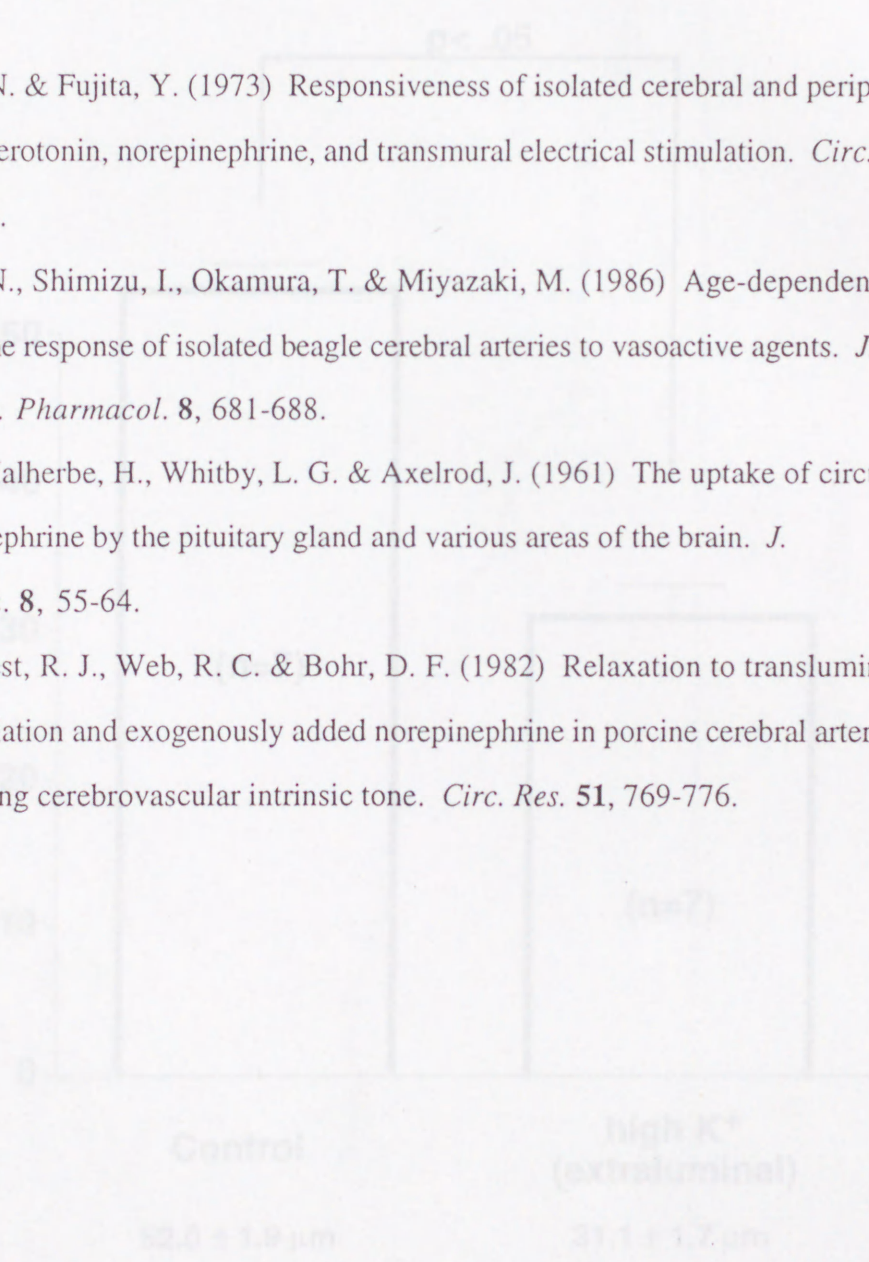
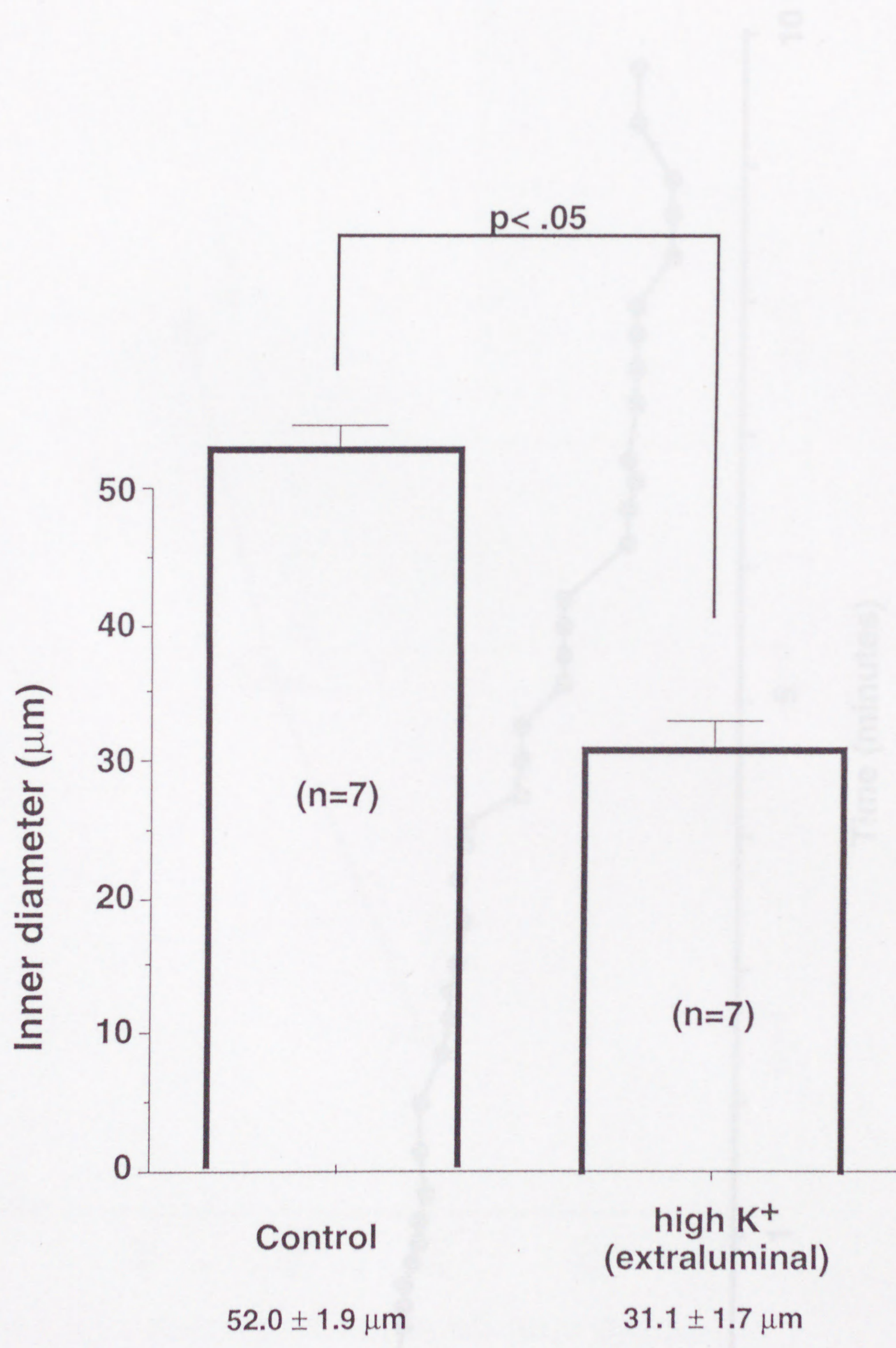


Fig 1



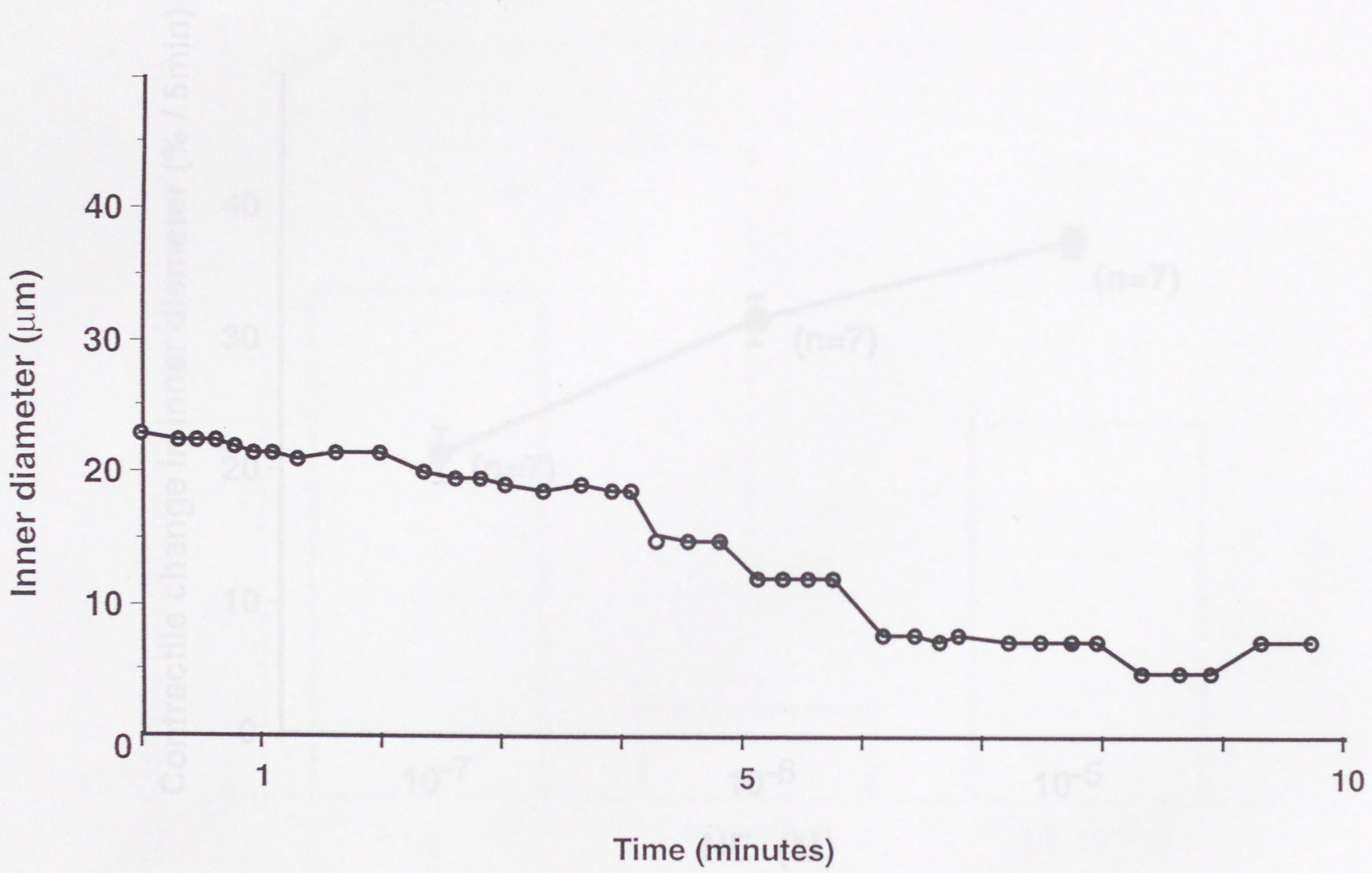
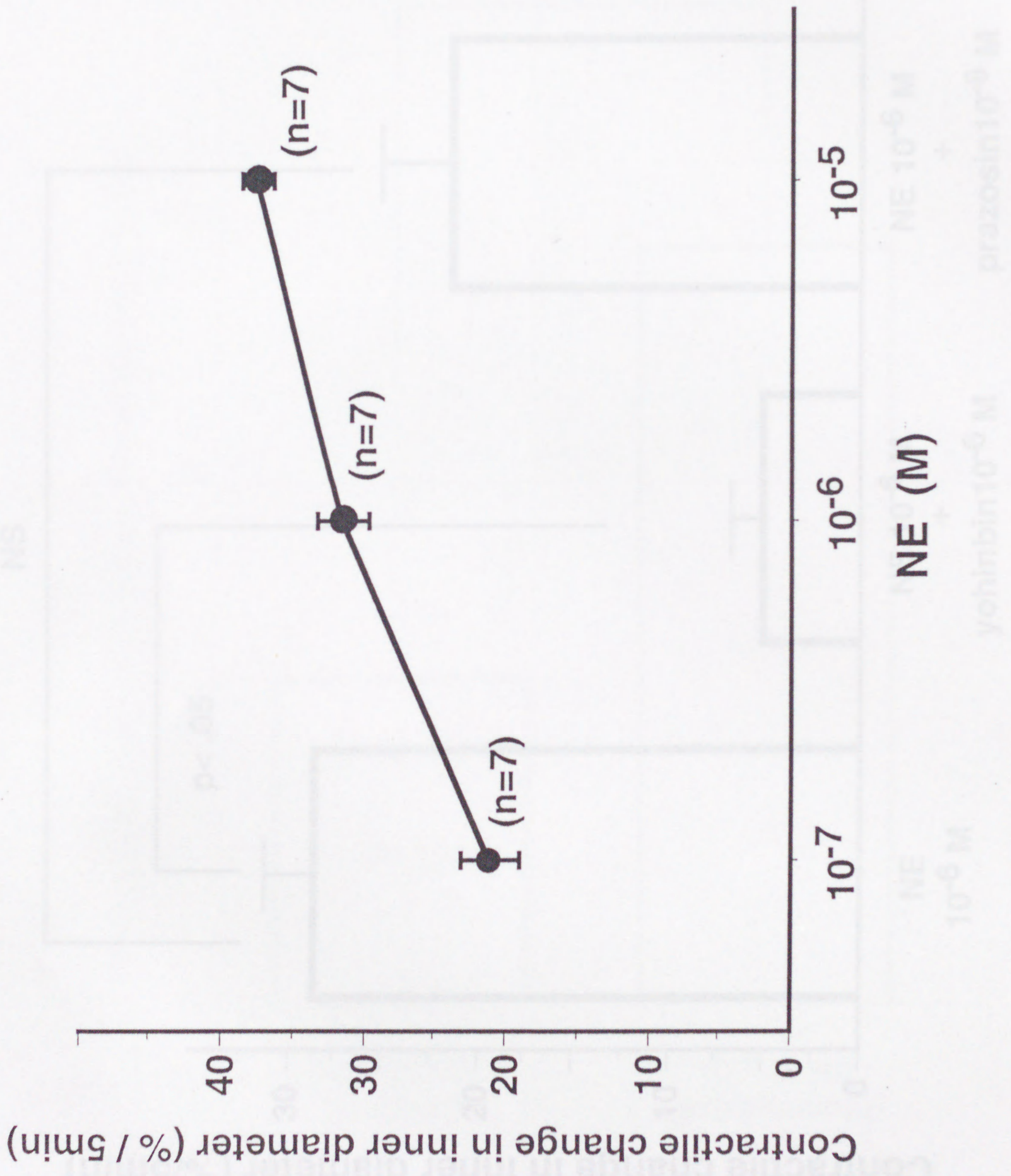


Fig.2

Fig. 3



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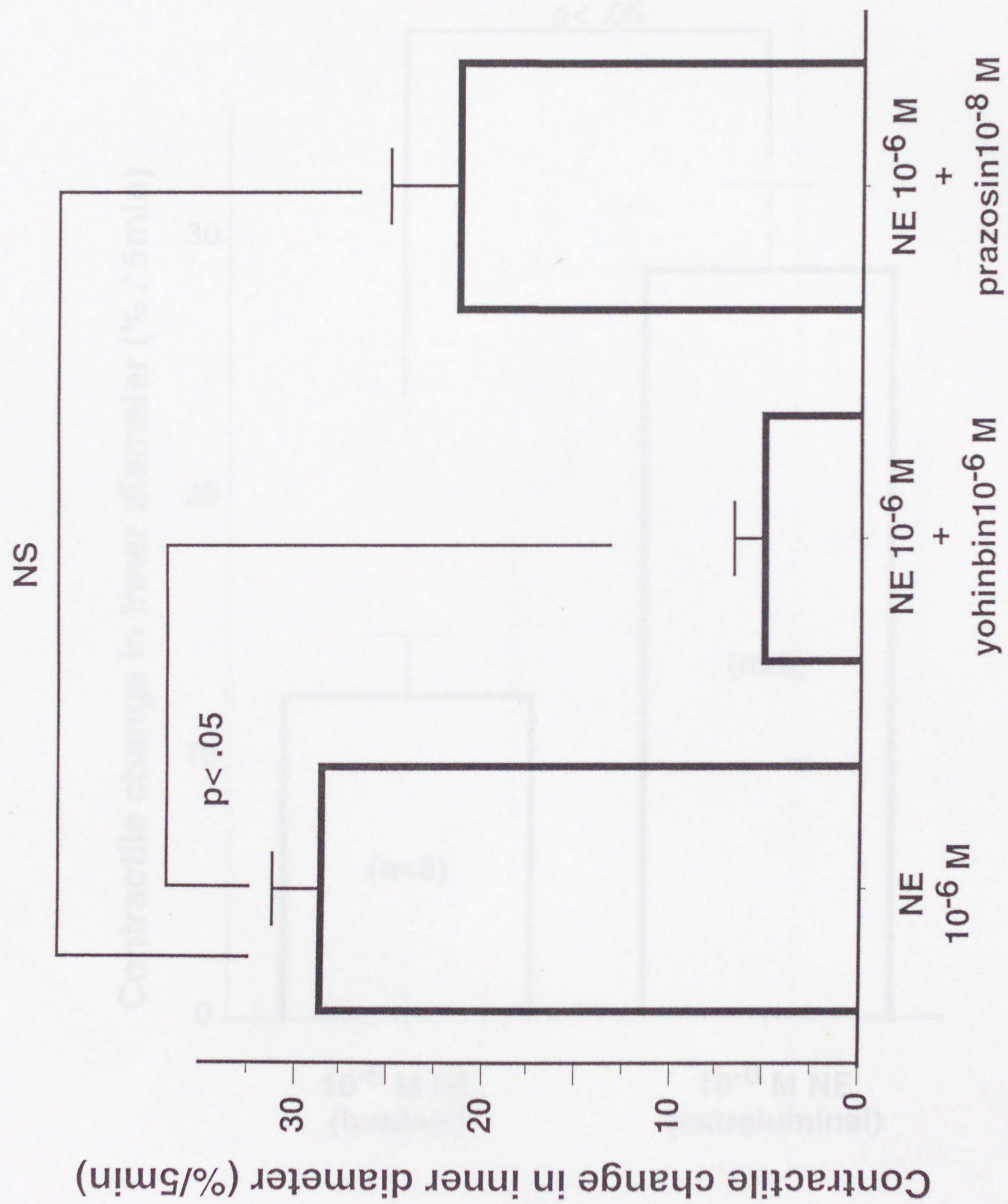
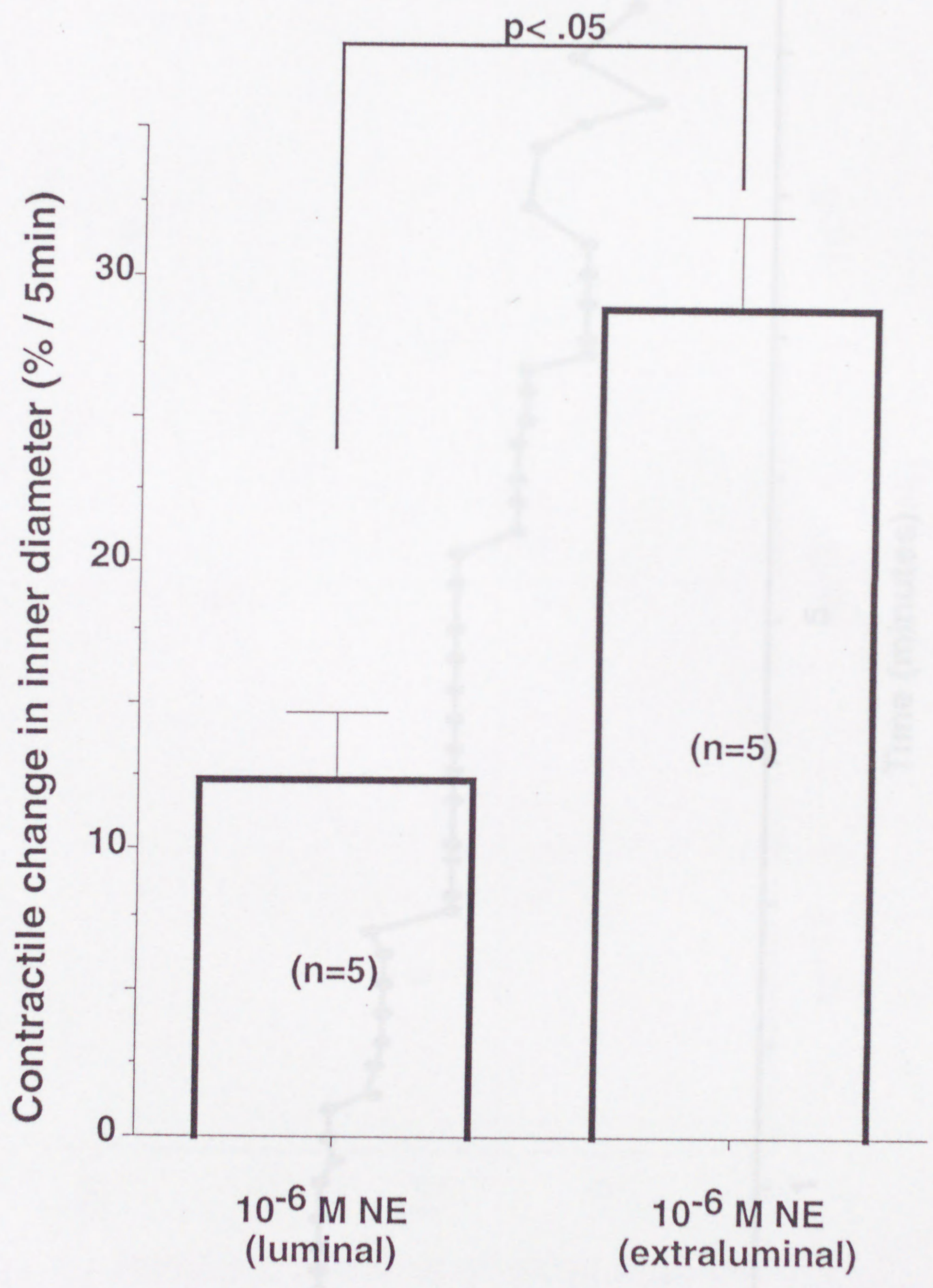


Fig 5



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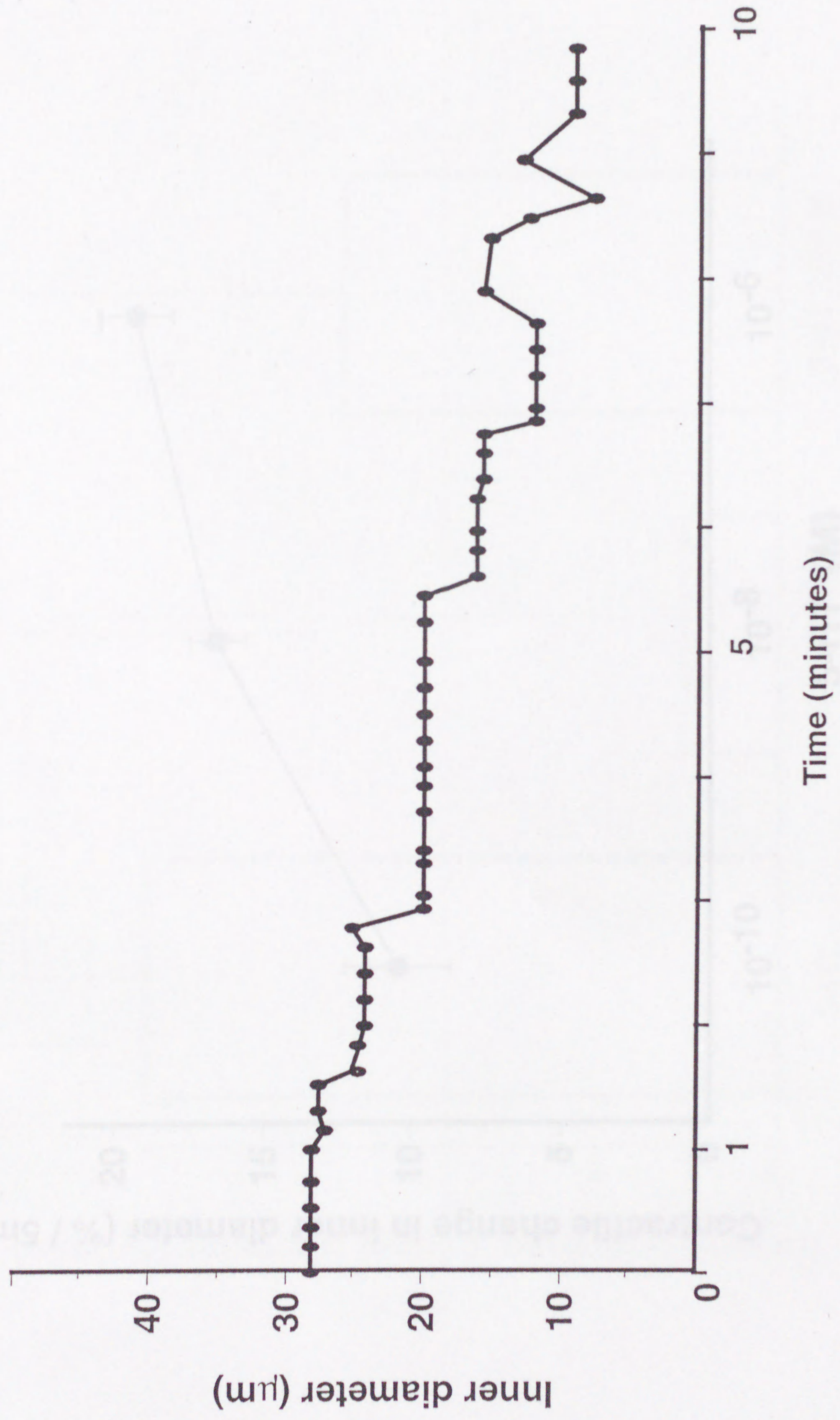

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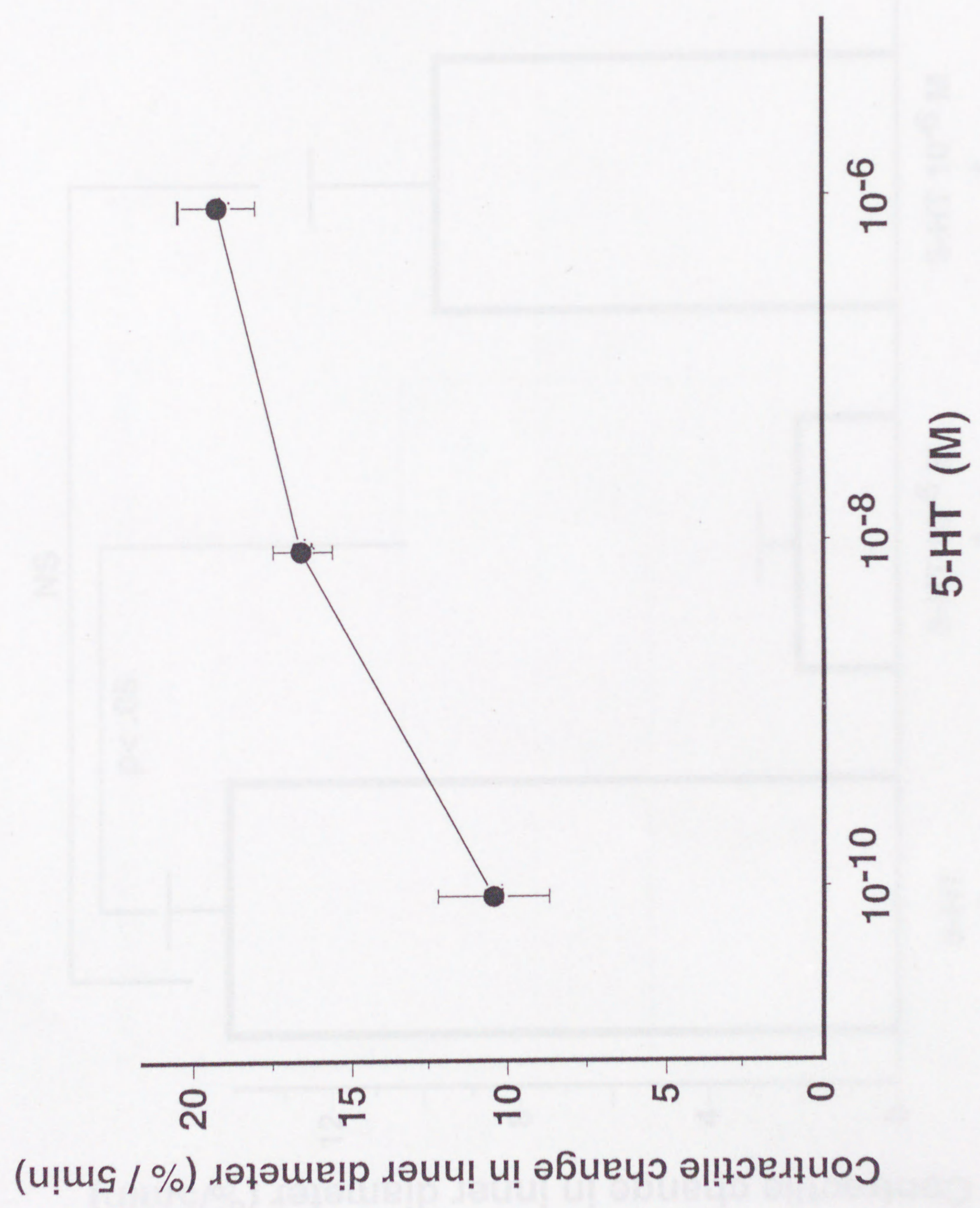

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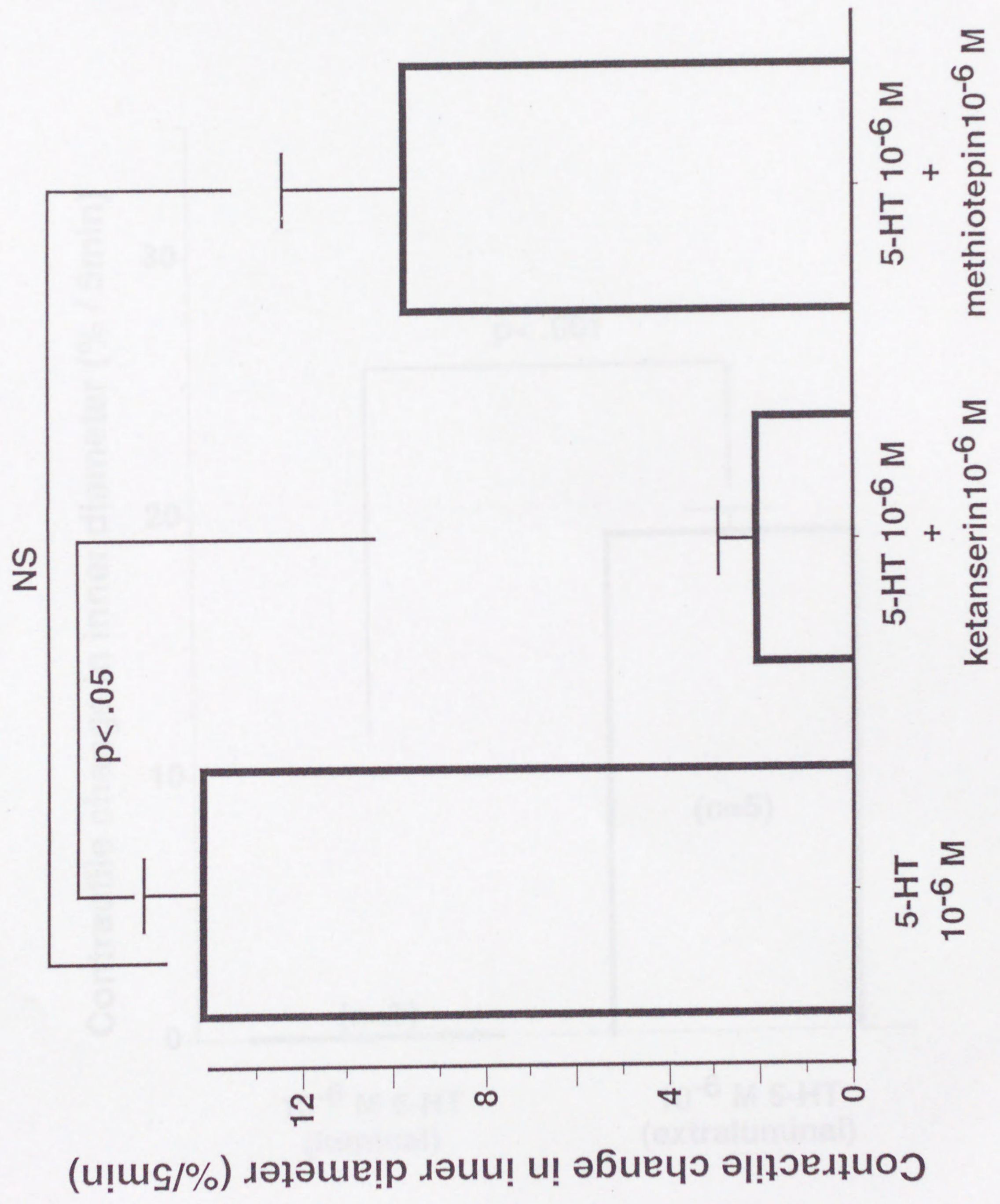

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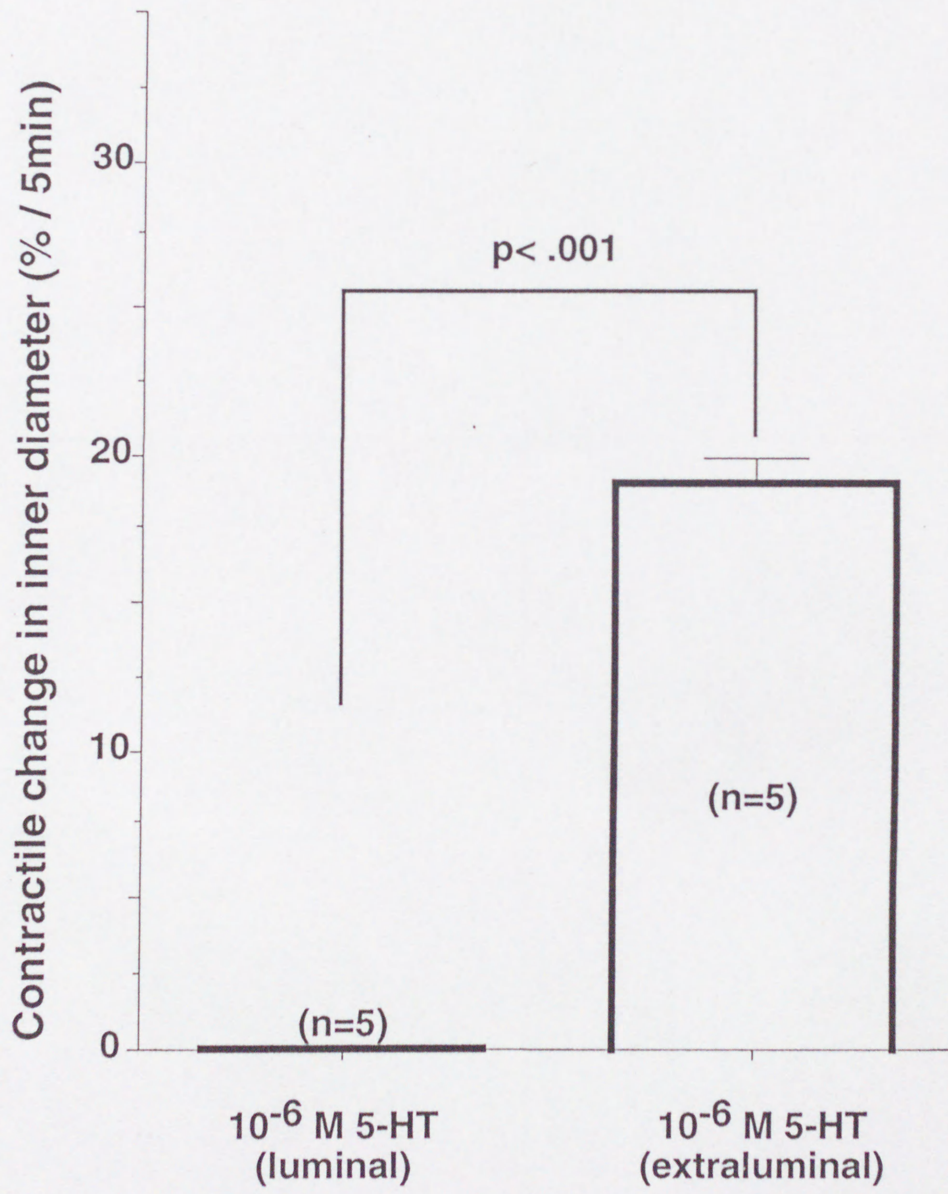

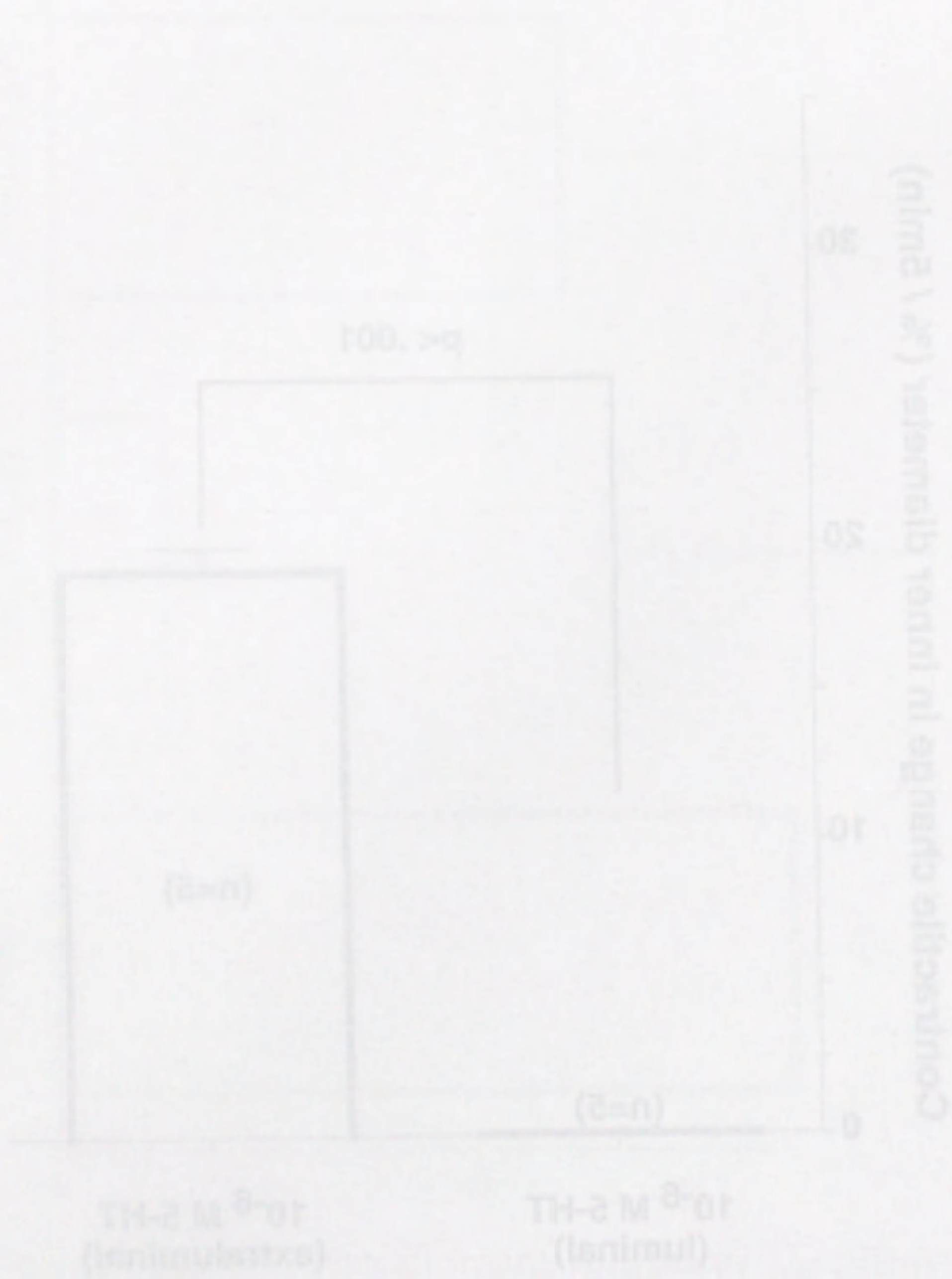

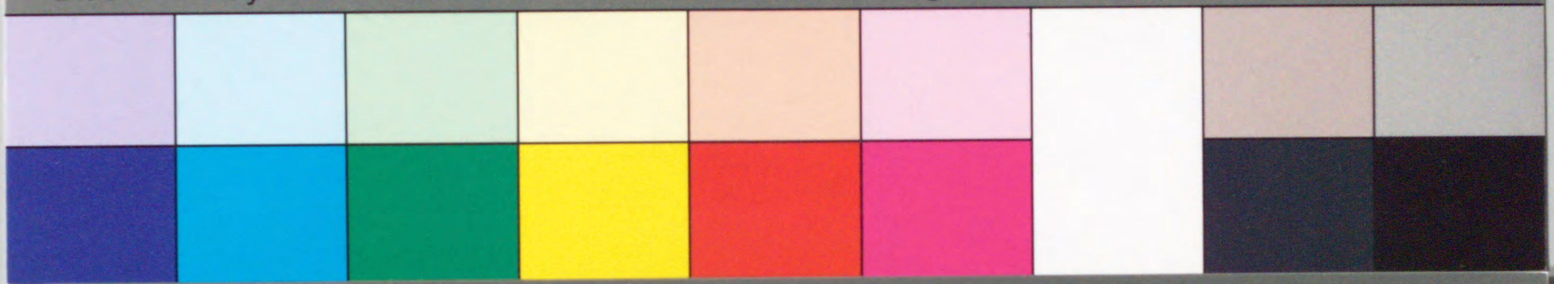

Kodak Gray Scale

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A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19