Smullin, David Hyam

TEMPERATURE REGULATION: CENTRAL NEUROLOGY AND THE ROLE OF GAMMA-AMINOBUTYRIC ACID

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TEMPERATURE REGULATION: CENTRAL NEUROLOGY AND THE ROLE OF GAMMA-AMINOBUTYRIC ACID

A

THESIS

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TEMPERATURE REGULATION: CENTRAL NEUROLOGY AND THE ROLE OF GAMMA-AMINOBUTYRIC ACID

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ABSTRACT

This thesis investigates the hypothesis that thermoregulation may depend upon opposing responses of hot and cold temperature sensors with reciprocal inhibition between the efferent signals to the heat loss and heat production effectors, rather than upon comparison of a regulated variable with a temperature insensitive reference signal. A physical model was built to demonstrate that temperature regulation can work on this principle, and intracerebroventricular injections (ICV) of synaptically active substances were made into sheep to investigate the role of gamma-aminobutyric acid (GABA) as a neurotransmitter of reciprocal inhibition in thermoregulation.

The model consisted of two inversely-related temperature-dependent signal generators connected to opposing correction effectors which served to heat and cool a plexiglass chamber. Reciprocal inhibition between the efferent pathways created a thermoregulatory null-zone which could be varied by manipulating signals converging onto either pathway to qualitatively simulate physiological responses to fever, hibernation and ICV injections of synaptically active substances.

ICV injections of GABA or its agonist muscimol inhibited heat loss in the heat and heat production in the cold in sheep. An ICV injection of a GABA blocker prior to the ICV injection of an excitatory transmitter of either the heat production pathway in the heat or the heat loss pathway in the cold activated both heat production and heat loss effectors

simulataneously.

These results support the hypotheses that thermoregulation may depend upon opposing responses of sensor signals with reciprocal inhibition between the signals to opposing effectors and that GABA acts as the neurotransmitter of reciprocal inhibition.

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ACKNOWLEDGEMENTS

A dissertation has only one author, but rarely is it the product of a single person. This is especially so in my case, and I am deeply indebted to a number of people without whose assistance this project would never have been successfully completed.

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Dedicated to the memory and inspiration of my brother

Frank M. Smullin

PREFACE

The nature of the interface of the temperature sensors and thermal effectors in the mammalian central nervous system is a controversial topic. In the past many, if not most, investigators of thermoregulation have chosen to ignore this topic and considered the interface a "black box". Other investigators took their clue from the engineers and assumed that since both animal and mechanical thermoregulatory systems appear to regulate temperature in a similar manner the animal system must employ a temperature insensitive reference signal against which core temperature is compared. This notion gained wide acceptance and is still popular today but is based on little physiological evidence. Is the physical system of thermoregulation employed by engineers the most likely system to be employed by animals or do simpler models exist that are supported by physiological evidence and are similar to other systems of physiological integration and regulation?

An exciting subject in the field of thermoregulatory physiology deals with the pharmacology of the central interface between the thermal sensors and effectors. John Bligh and his colleagues have carried out an extensive investigation of this area using the sheep (see Bligh 1979). A simple neuronal model that did not employ the engineer's reference signal but which depended upon the interactions of opposing sensor signals with reciprocal inhibition between the efferent signals to opposing effectors, was developed from the results of these studies. This model adheres to the

principles for integration of information in the central nervous system originally set forth by Sherrington (1906). These principles as summarized by Bligh (1984) are: 1) that every sensor to effector pathway contains at least one neuronal synapse; 2) that every synapse acts as a signal mixer, receiving excitatory and inhibitory influences from elsewhere in the central nervous system, as well as from a dominant sensor; 3) that this signal mixing means that no sensor/effector relations are fixed and invariable; and 4) that where two effector functions act in opposition to each other, the opposition is neutralized by reciprocal inhibition between the two efferent pathways. Thus, this is a model that is based on physiological evidence and adheres to basic principles of physiological integration.

In this dissertation I will address two different, but related, aspects of this model. I will demonstrate that this model can simulate some of the basic properties of mammalian thermoregulation and that a physical system for thermoregulation can function without a reference signal. I will also present pharmacological evidence supporting the hypothesis that the inhibitory amino acid, gamma-aminobutyric acid (GABA), is the neurotransmitter of reciprocal inhibition between the two thermoregulatory pathways of the model. An important aspect of this study is that rather than attempting to produce a model of a biological system based on engineering principles, both a physical and a neuronal model have been produced from biological evidence.

CHAPTER 1

A PHYSICAL MODEL OF THERMOREGULATION BASED ON A NEUROPHYSIOLOGICAL THEORY

Introduction

Temperature regulation of mammals and other vertebrates has been one of the most thoroughly studied areas of animal physiology. The physiology and anatomy of the thermoregulatory effectors and thermosensors and the characteristics and identification of many adaptations for thermoregulation have been studied in detail and are well understood.

The relative ease of measurement and manipulation of temperature have been partially responsible for the degree of attention and resultant knowledge of these peripheral aspects of temperature regulation. In contrast, although the interface in the central nervous system between the sensory and effector mechanisms of thermoregulation have been well defined anatomically, little is known of these neurological pathways beyond the theoretical stage, partially due to the inherent complexity of the vertebrate central nervous system.

There is apparently a widespread notion that neuronal models, in general, are too simplistic to accurately describe the complex interactions of the central nervous system. Because of the actual complexity, coupled with this notion that it is hopeless to attempt to define any other neurological pathways, this interface has been depicted as a black box in many models of temperature regulation. Unfortunately this argument and its nonproductive conclusion are apparently made in complete ignorance of the definition and role of models. MacDonald and Wyndham (1950) state that model building is done: a) to reduce the actual subject for analysis to

sufficiently few elements for a mathematical (or experimental) treatment of its behavior under any desired condition to be possible, and b) to crystallize in one diagram (or piece of apparatus) the characteristics of a system which otherwise requires complex mathematical and verbal explanations. Dainty (1960) defined the role of a model even more succinctly in the following statement, " Thus an analog both simplifies and puts into familiar terms a complicated phenomenon and hence enables one to think more clearly about the subject." Boulant (1981) points out that, " Regardless of whether a neuronal model is correct or incorrect, it can still serve a useful purpose. A model can explain complicated, often confusing data in a way that can be easily understood by the scientific community. A model presents a clearly defined hypothesis that can be tested by a variety of techniques from several different laboratories. In this way, neuronal models stimulate experimentation as well as communication among laboratories." Finally, Kac (1969) stated: " Models are, for the most part, caricatures of reality, but if they are good, then, like good caricatures, they portray, though perhaps in a distorted manner, some of the features of the real world."

In a review of mathematical and physical models of thermoregulation Hardy(1972) defines and discusses the major characteristics and differences in the various kinds of mathematical and physical models available for use in depicting thermoregulation. Bligh (1973) made an extensive review of many of the engineering and neuronal models of thermoregulation. In these reviews a fundamental point in control theory is made; this is the division between the "controlled system" and the

"controller" . In temperature regulation of vertebrates the controlled system usually refers to the internal temperature or heat content of the body, and the controller is the neuronal system, apparently centered in the hypothalamus. In the literature engineering and mathematical models of the controlled system are far more numerous than those of the controller. Models of the controlled system range from the simple electrical analog of MacDonald and Wyndham (1950) to the incredibly complex mathematical model of Wissler (1964). Increasing complexity in a model may add to the accuracy with which the model depicts the response of the modeled system to disturbances in the real world. However, the same complexity that increases the accuracy of the model also vastly complicates the analysis and understanding of the system as the complexity of the model approaches the reality of the system. Thus great complexity and accuracy in a model may defeat the reason for producing models as defined by Dainty (1960), and the complex model may fail to produce one of the goals of model-building set forth by Boulant (1981); that of stimulating further research and discussion.

In this chapter I will concentrate on neuronal and chemical models of the thermoregulatory controller in the hypothalamus. I will also present a physical model of temperature regulation based on Bligh's neuronal model of thermoregulation in the sheep (1979) in which the set-point temperature is established by the inherent properties of the opposing pathways from cold and hot thermosensors to the heat production and heat loss effectors, respectively. The set-point in this model does not depend on any form of stable reference signals.

Traditionally the mammalian thermoregulatory system has been considered in terms of engineering systems of temperature regulation. In such a system the controlled variable (i.e. core temperature) is compared with a fixed reference signal, producing an error signal that activates either the heat production or heat loss effector functions, dependent on the degree and direction of error. In the case of mammalian thermoregulation the stable reference signal would presumably be produced by temperature insensistive central neurons (Fig 1.1a). This type of model can accurately predict the responses of the mammalian thermoregulatory system to changes in ambient conditions and thermal load of the system. With the addition of external synaptic inputs onto the stable reference signal the model can describe the effects of fever and exercise which appear to shift the set-point. Although the reference signal model of the engineered system is capable of predicting the responses of a thermoregulatory system there is little experimental neuronal evidence, other than the existence of temperature insensitive neurons in the hypothalamus, to suggest its accuracy as a neuronal model.

Bazett (1927) was the first to suggest the existence of warm and cold sensitive neurons involved in temperature regulation. He further suggested a tentative hypothesis, unsupported by experimental evidence at the time, that if these neurons possessed different activity/temperature characteristics they could form the basis of temperature regulation (Fig 1.1b). Vendrik (1959) interpreted and expanded on Bazett's proposal by hypothesizing cold and warm sensors with bell-shaped activity/ temperature curves with the cold sensor activity peak at a lower

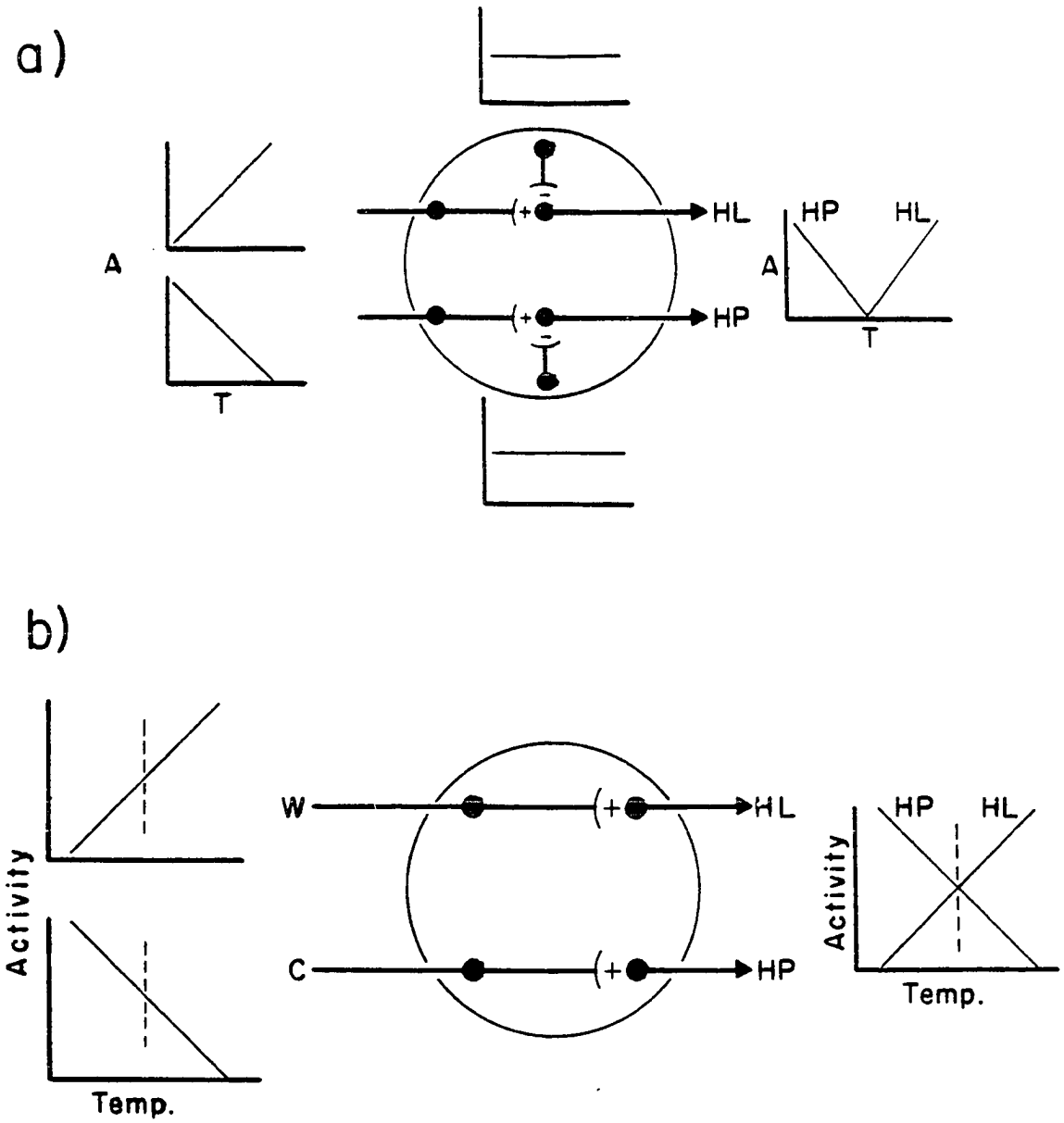

Fig 1.1. a) The classical engineering model of a thermoregulatory system in which the regulated temperature is compared with a fixed reference signal to produce an error signal that activates either the heat loss (HL) or heat production (HP) effectors, dependent on the degree and direction of error. b) The hypothesis of Bazett (1927) and Vendrik (1959). Cold and warm sensors have reciprocal activity/temperature responses with the activity peak of the cold sensors at a lower temperature than that of the warm sensors, producing a set-point temperature at the intersection of the two curves.

temperature than that of the warm sensor. If the homeostatic temperature is at the point where these curves intersect, then this point would have a domain of attraction creating a stable equilibrium. The sensors would then contain the properties necessary to produce a set-point without the need of a reference signal. Hensel and Zotterman (1951) had previously shown that cold and warm receptors in the tongue of the cat have activity/temperature curves similar to those proposed by Vendrik.

Since the time that these early conceptual hypotheses were proposed, based on very little evidence, a large amount of supportive experimental evidence, mostly from unit activity studies of thermoresponsive neurons in the hypothalamus, has been produced. Nakayama *et al* (1963), Eisenman and Jackson (1967), Hellon (1967) and Boulant (1981) obtained single unit recordings of cells in the preoptic/anterior hypothalamus (PO/AH) of cats and rabbits some of which appeared to be warm sensitive and others cold sensitive. Cabanac *et al* (1968) found warm and cold sensitive neurons in the PO/AH of the rabbits that possessed the bell-shaped curves with different peak activities as shown previously in the tongue by Hensel and Zotterman (1951) and proposed for the brain by Bazett (1927) and Vendrik (1959). Edinger and Eisenman (1970) located warm and cold sensitive units in the posterior hypothalamus, a site also hypothesized to be involved in central temperature regulation. It is interesting to note, although not necessarily directly significant to this discussion, that Boulant (1981) presents evidence suggesting that the response of cold sensitive neurons to cooling may be due to removal of inhibitory synaptic inputs from nearby

warm sensitive neurons onto spontaneously firing temperature insensitive interneurons, thus producing a cold sensitive unit.

Since the proposals of Bazett and Vendrik a number of models of temperature regulation have been developed that produce a set-point without the use of a reference signal. The basis of thermoregulation in almost all of these models has been the reciprocal intersecting responses of the heat production and heat loss pathways coupled with a feedback loop of the circulating blood to the central controller located in the hypothalamus. Smith *et al* (1964), Cornew *et al* (1967) and Atkins and Wyndham (1969) have created working analog models of the human thermoregulatory system based on these principles. These models did not include neuronal models depicting the mechanisms of the controller. Rather, the controller was depicted as a black box with given disturbance/response relationships which mimic the relationships between core temperature and skin temperature on the one hand and heat production and heat loss mechanisms on the other.

Three important neuronal models encompassing the Bazett/Vendrik proposal have been developed. Each one of these models was based on different forms of experimental evidence: 1) Wyndham and Atkins (1968) generated a model (Fig 1.2a) based on their analysis of the relations between thermal disturbances and the thermoregulatory responses of nude man; 2) Hammel (1965) produced a model (Fig 1.3a) based on studies of the electrical activities of single neurons in the PO/AH, and 3) Bligh (1973) developed a model (Fig 1.4) based on synaptic interference studies. When these three models were redrawn in the same format (Fig 1.2b, 1.3b and

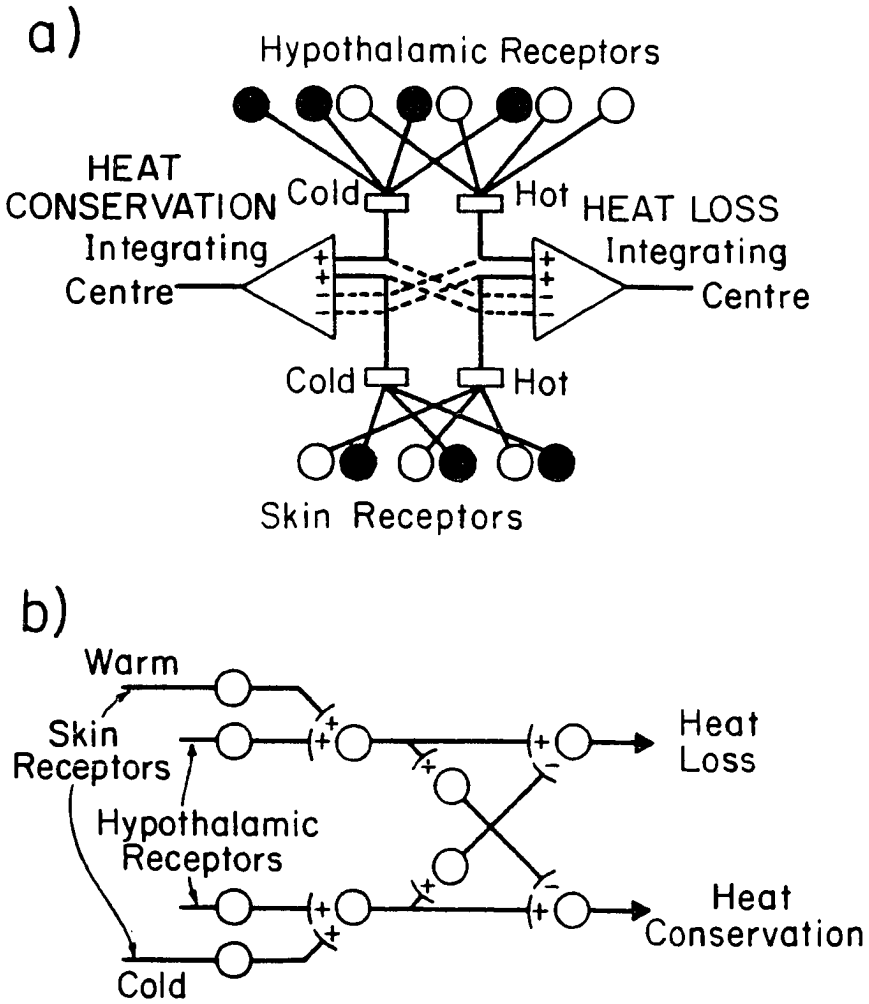

Fig 1.2. a) A diagramatic representation of the relations between hypothalamic and skin temperature sensors proposed by Wyndham and Atkins (1968). b. The same relationship as redrawn by Bligh (1973).

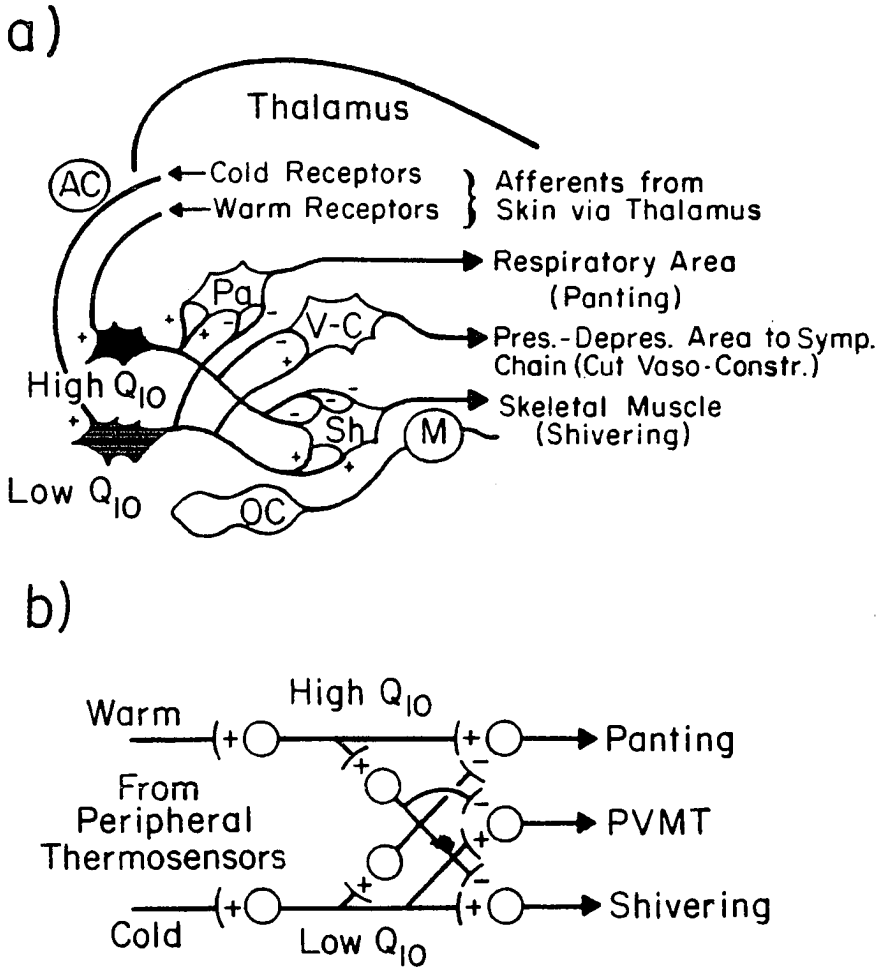

Fig 1.3. a) Relations between peripheral warm and cold sensors and the hypothalamic "high Q_{10}" and "low Q_{10}" neurons as depicted by Hammel (1965). b) The same relationship as redrawn by Bligh (1973). PVMT = peripheral vasomotor tone.

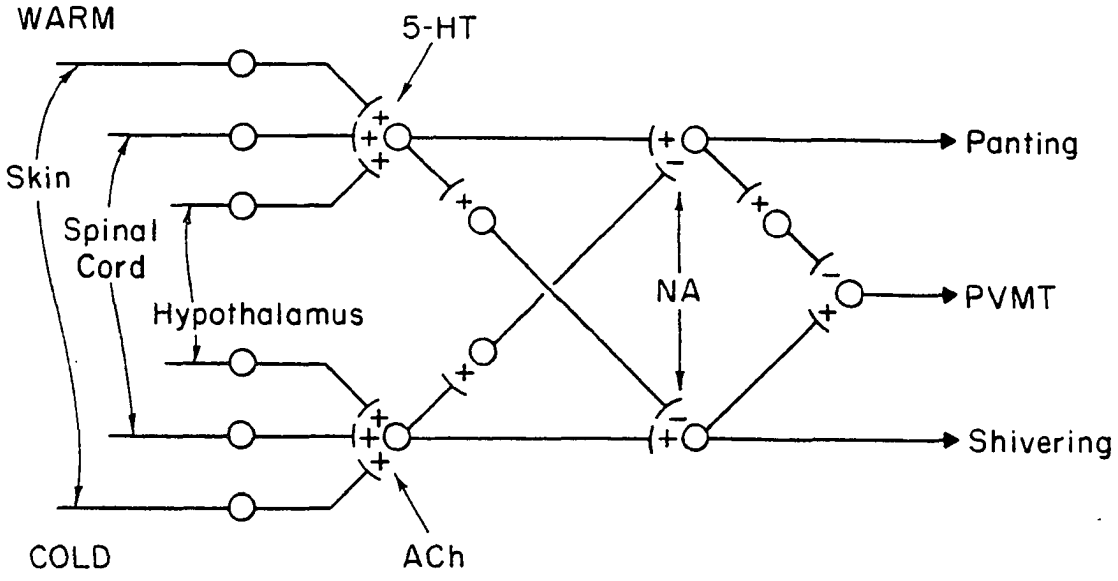

Fig 1.4. A neuronal model based on the synaptic interference studies of Bligh (1973). PVMT = peripheral vasomotor tone; 5-HT = 5-hydroxytryptamine; NA = noradrenaline; ACh = acetylcholine.

1.4), and analyzed and compared by Bligh (1973) they were shown to be functionally similar and to all contain three basic characteristics: 1) each consists of two main opposing multisynaptic pathways; one from central warm sensors to heat loss effectors, and the other from central cold sensors to heat production effectors; 2) they each have reciprocal inhibitory pathways between these two main pathways, and 3) neural pathways from peripheral temperature sensors converge onto the two main sensor to effector pathways to modify the drive from central sensors and to allow for a variable set-point. On further analysis it is apparent that these characteristics form the basic principles for the integrative action of the central nervous system originally proposed by Sherrington (1906) 80 years ago (Fig 1.5).

The three neuronal models of thermoregulation described above produce the same results (Fig 1.6) as the model shown in Figure 1.1a, and appear to do so with the simplest neuronal arrangement necessary to allow for regulation. In spite of this, as far as I have been able to determine there have been no physical systems for regulating temperature built on the basis of the Bazett/Vendrik proposal. Thus a physical model of Bligh's neuronal model of temperature regulation was built, and in this chapter I will demonstrate that this system is capable of regulating the temperature of a small plexiglass box without the use of a temperature insensitive reference signal. Furthermore, I will show that this model can, with the addition of the appropriate external inputs, simulate the physiological changes in thermoregulation that accompany the onset of fever and hibernation. In chapter three I will demonstrate that this model can simulate the

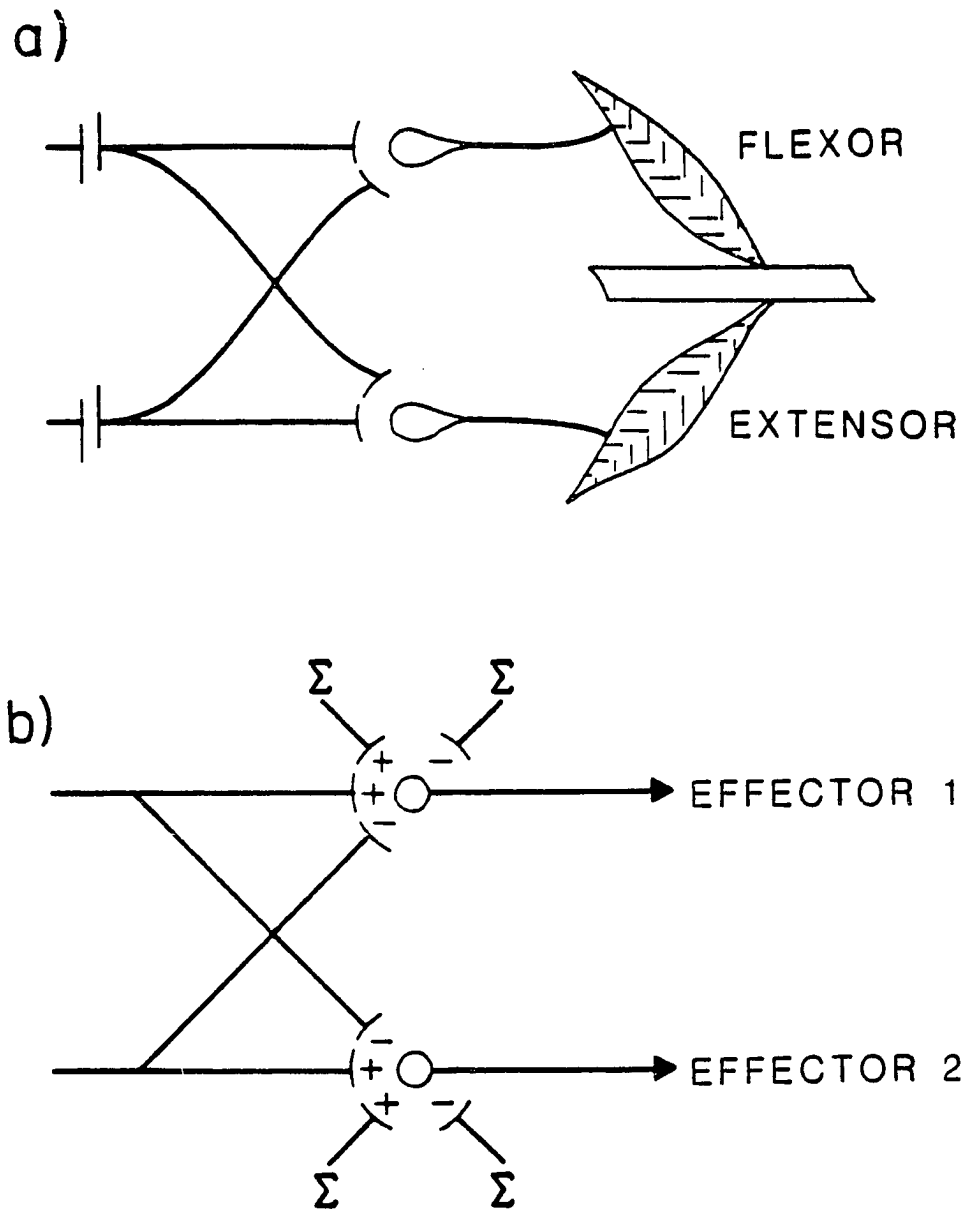

Fig 1.5. a) Reciprocal inhibition between the efferent pathways to antagonistic flexor and extensor muscles as depicted by Sherrington (1906). b) An interpretation of the Sherringtonian concepts of basic central neuronal organization, where Σ = summed excitatory (+) and inhibitory (-) influences acting on antagonistic effector pathways, between which there is also reciprocal inhibition.

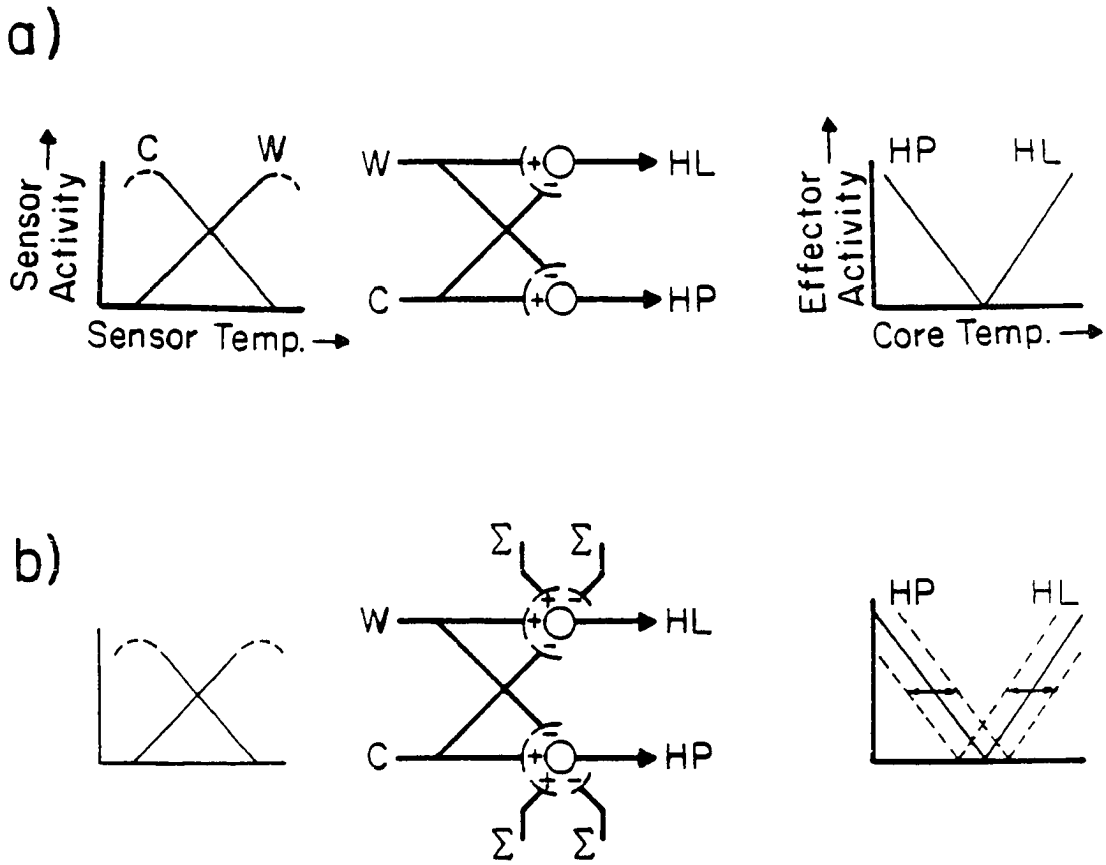

Fig 1.6. a) The simplest neuronal arrangement necessary to account for the relations between the thermosensor activities and temperature on the one hand, and those between thermoregulatory effectors and core temperature on the other hand (Bligh 1979). b) The addition of excitatory (+) and inhibitory (-) influences from elsewhere in the CNS could account for set-point variability (Bligh 1979).

pharmacological experiments described in chapter two.

Methods

Chamber

A chamber (0.1 m x 0.1 m x 0.15 m) was built of 6.35 mm plexiglass (k = 0.17 W/mK) covered with 16 mm foam insulation (k = 0.04 W/mK). One end of the chamber (0.1 m x 0.1 m) was left open to accommodate a thermoelectric peltier cooling device. The heat flow (q) of a completely enclosed box of the same dimensions was calculated using the formula:

1) $$q = (A \cdot (k/x) \cdot \Delta T)$$

$$= 0.18 \cdot \Delta T$$

q = heat flow (W)

A = surface area of chamber (m^2)

k = thermal conductivity (W/mK)

x = thickness of material (m)

$\Delta T = T_{in} - T_{out}$

T_{in} = temperature inside of chamber (K)

T_{out} = temperature outside of chamber (K)

Assuming a normal T_{in} of 33° C; at T_{out} = 30° C, q = 0.54 W; and at T_{out} = 35° C, q = 0.36 W.

Temperatures

The insulated chamber was tightly wrapped in thin walled tygon tubing held in place by two layers of duct tape. The tygon tubing was attached to a temperature controlled water bath, which pumped water at a set temperature through the tubing. The temperatures at the interface of

the tubing and insulation and inside the chamber were measured with thermocouples and were considered to be T_{out} and T_{in} respectively.

Basal Metabolic Rate

Without performing heatflow calculations for the aluminum plate and copper cooling fins of the peltier device, I estimated the heatflow of the chamber with the cooling device in place to be at least 2.5 W at T_{out} = 30° C. Thus in order to include a basal metabolic rate (BMR) in the model, and to allow regulation within a 5° C range of the arbitrary 33° C set point without needing a large input of power, a 10 Ω, 10 W resistor in line with a 5 V power source was installed in the chamber. The inclusion of this circuit in the chamber acted as a constant 2.5 W BMR.

Temperature Sensors

Two AD592CN IC temperature transducers (Analog Devices) with linear thermal response characteristics were used as temperature sensors. By use of circuits built around high performance 741 operational amplifiers (Fig 1.7) these sensors were given reciprocal temperature response outputs of 0-10 V over a 7° C temperature range, thus producing a cold sensor and a heat sensor (Fig 1.8). The outputs of these circuits were measured with voltmeters and the voltages were recorded on a two-channel strip chart recorder.

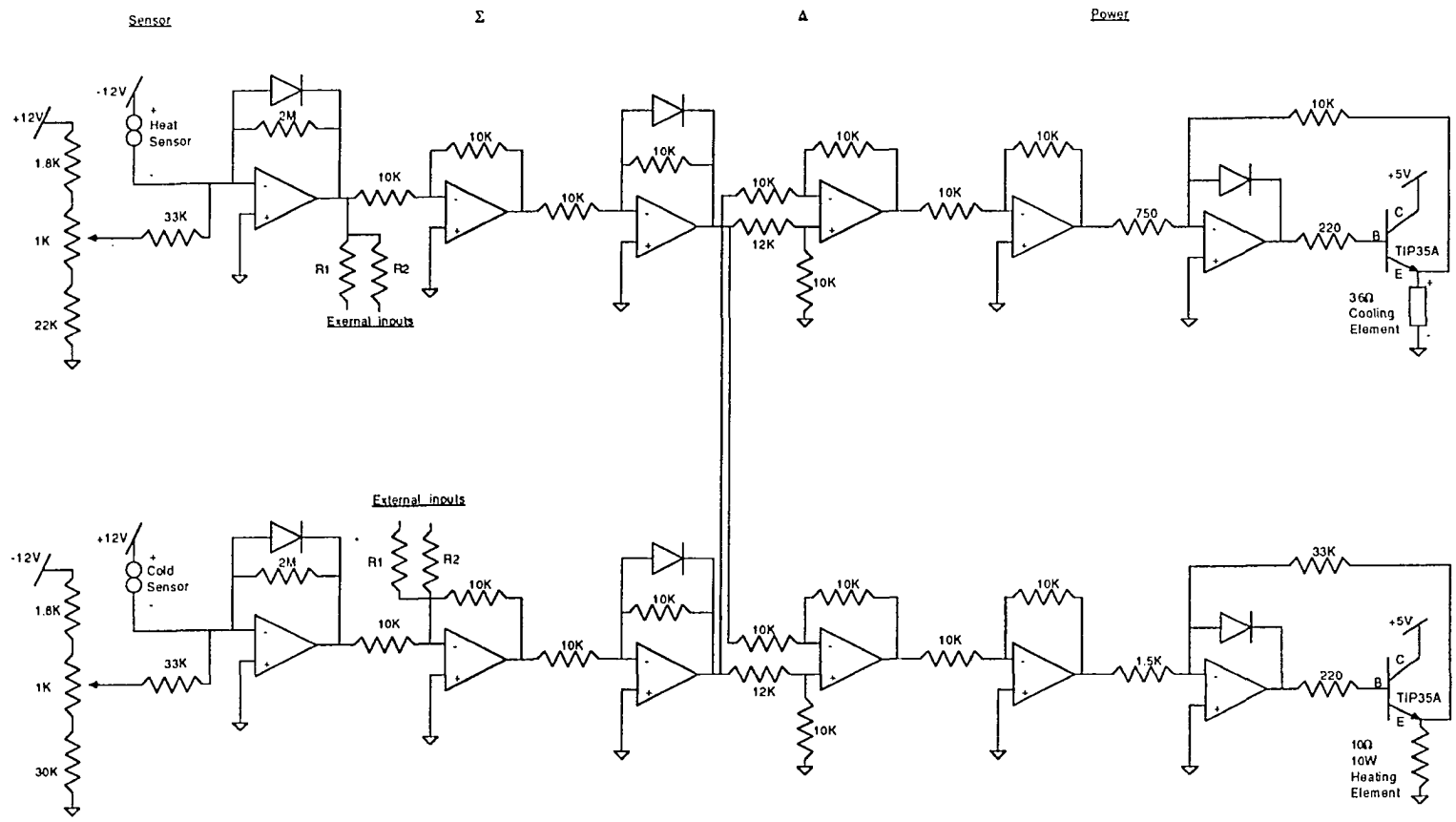

Fig 1.7. Schematic of the electronic circuitry of the physical model. All operational amplifiers are high performance 741s. Σ = summer, Δ = differencer.

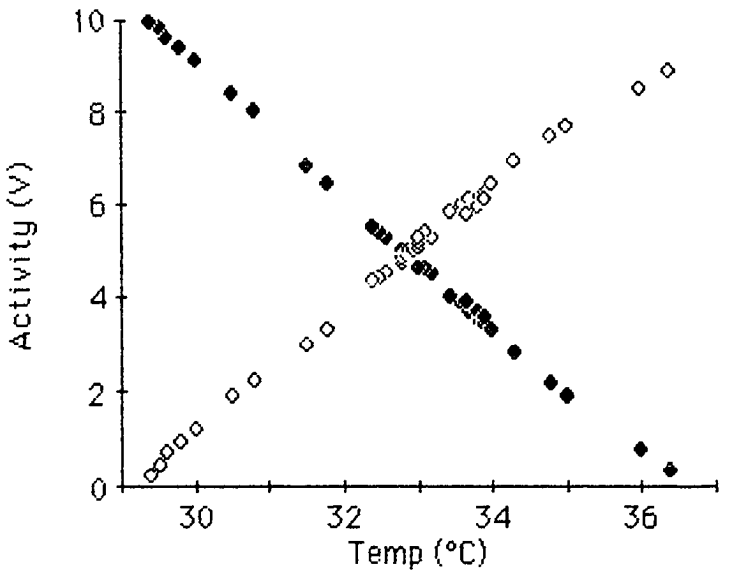

Fig 1.8. Activity versus temperature curves of the temperature sensors. Open circles (o) indicate heat sensors. Closed circles (●) indicate cold sensors.

Heat Production Effector

The variable heat source in the model was a 10 Ω, 10 W power resistor, similar to that used for the BMR. The circuitry (Fig 1.7) connecting this heat source to the sensors produced a proportional controller with an output range of 0-2.4 W for an input of 0-0.5 V. The output of this circuit was measured with a voltmeter and the voltage was recorded on a two-channel strip chart recorder. The power was calculated from the formula:

2) $$P = V^2/R$$

P = Power (W)

V = Potential (V)

R = Resistance (Ω)

Heat Loss Effector

A thermoelectric peltier cooling device was used in the model as the heat loss effector. This device had a nominal resistance of 3.6 Ω and the amplifier circuit (Fig 1.7) produced a proportional output of 0-6.4 W for a 0-0.8 V input from the sensors. The output of this circuit was measured with a voltmeter and the voltage was recorded on a two-channel strip chart recorder. Power was calculated as described above.

Circulation

A 4-blade 9 mm diameter aluminum fan, driven by a small external electric motor, was used to circulate air within the chamber. The fan was mounted 30 mm above the bottom of the chamber, and was able to produce a uniform T_{in}.

Block Diagram

A block diagram of the model (Fig 1.9) consists of two opposing pathways; one from cold sensor to heat production effector, and one from heat sensor to heat loss effector, with reciprocal inhibition between the two pathways. Each of the pathways consists of four stages; 1) a temperature sensor, 2) a summer (Σ), 3) a differencer (Δ) and 4) a power source.

Schematic

Each of the four stages was designed and built around high performance 741 operational amplifiers (Fig 1.7). The sensor circuits were described above. The Σ stage, with unity gain, received the output from the appropriate sensor circuit (i.e. the Σ stage of the heat production pathway received the output of the cold sensor, while the Σ stage of the heat loss pathway received the output of the warm sensor). External inputs, representing environmental or internal disturbances, into either Σ (Fig 1.9) modulated the activity of both effectors. In this way hypotheses on the mechanisms of naturally occurring disturbances and modulations to thermoregulation, such as fever, could be tested with this model.

The Δ stage received the output of both Σ stages. A positive signal was received from one sensor to effector pathway while a negative signal was received from the reciprocal pathway. No signal was passed to the power stage of a pathway unless the positive input was greater than the reciprocal negative input. The gain of the inverting input was unity while

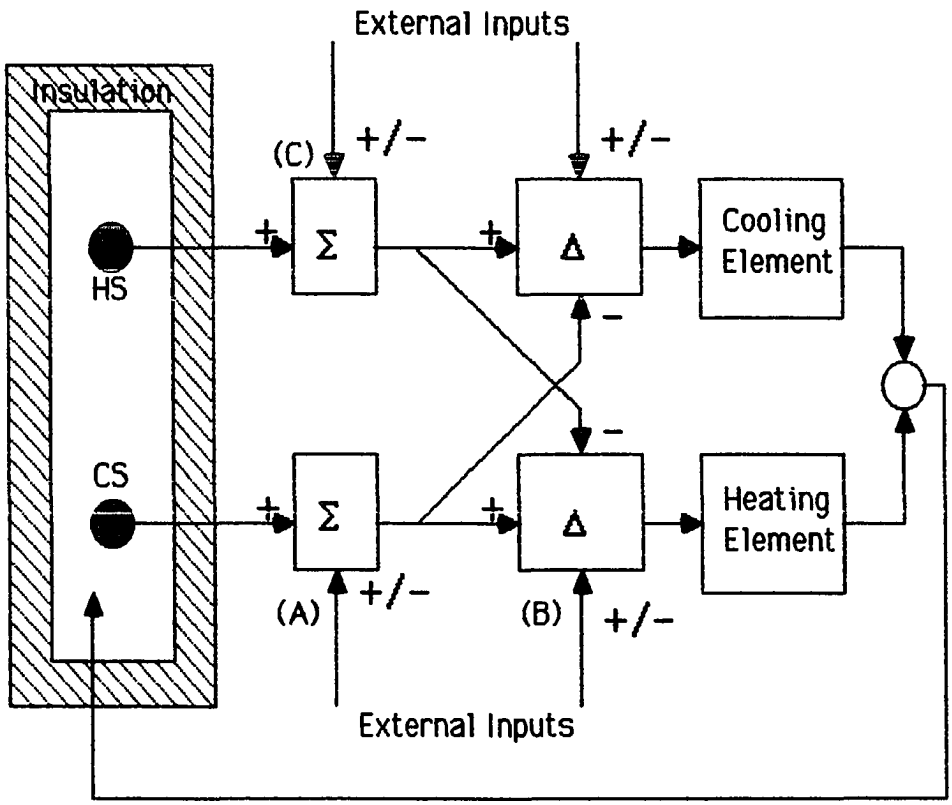

Fig 1.9. Block diagram of the physical model. HS = heat sensor, CS = cold sensor, Σ = summer, Δ = differencer, Heating element = 10Ω, 10W resistor, cooling element = thermoelectric peltier device, (+) = excitatory input, (-) = inhibitory input. A, B and C indicate points referred to in the text.

the gain of the noninverting input was 3.05, thus creating a null-zone over which both power stages were inactive. The Δ stage also received positive and negative signals representing external disturbances (Fig 1.9) modulating the null zone of the effectors. The external inputs to the differencers had no effect on reciprocal inhibition, and thus a disturbance to one Δ only modulated the activity of one of the effectors.

The power stages of the two pathways drove the two thermal effectors; 1) the power resistor used as a heat source, and 2) the peltier device used to cool the chamber. The circuits driving the heat source and cooling device had gains of 22 and 13 respectively (Fig 1.7).

Results

The set-point of this model was not a single temperature point but a zone ranging from 32.7° C to 33.0° C (Fig 1.10). Above and below this range the cooling and heating effectors, respectively, became active (Fig 1.11). In this discussion set-point refers to the range of T_{in} over which both thermal effectors are inactive, and does not imply the involvement of a reference signal.

Figure 1.10 shows that the thermoneutral temperature (i.e. T_{out} at which the set-point is maintained without activating either the heating or cooling effectors) was about 32° C. Thus it can be seen from the figure that at T_{out} = 32°C the temperature remained within the set-point range irrespective of whether the effectors were connected to the system or not, and when they were connected they were not active.

At T_{out} = 30° C the heating effector maintained T_{in} within the set-point range. When the effectors were disconnected T_{in} dropped 0.3° C below the set-point in 10 min, and returned to the set-point immediately after the effectors were reconnected to the system (Fig 1.10).

At T_{out} = 35° C the cooling effector maintained T_{in} within the set-point range. When the effectors were disconnected T_{in} rose 0.5° C in 10 min. T_{in} returned to the set-point immediately after reconnecting the effectors to the system.

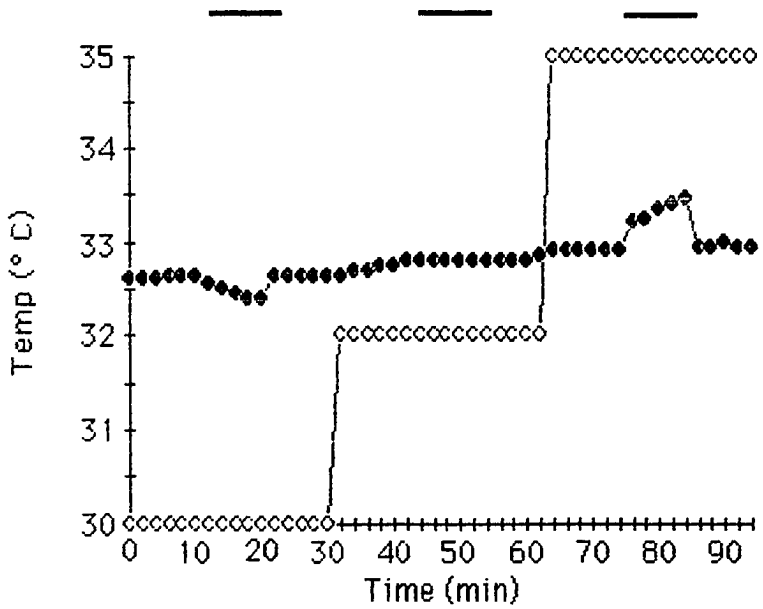

Fig. 1.10. Plot of T_{in} (●) and T_{out} (o) versus time. Solid lines above the graph indicate periods when the thermoregulatory effector functions were disconnected from the system.

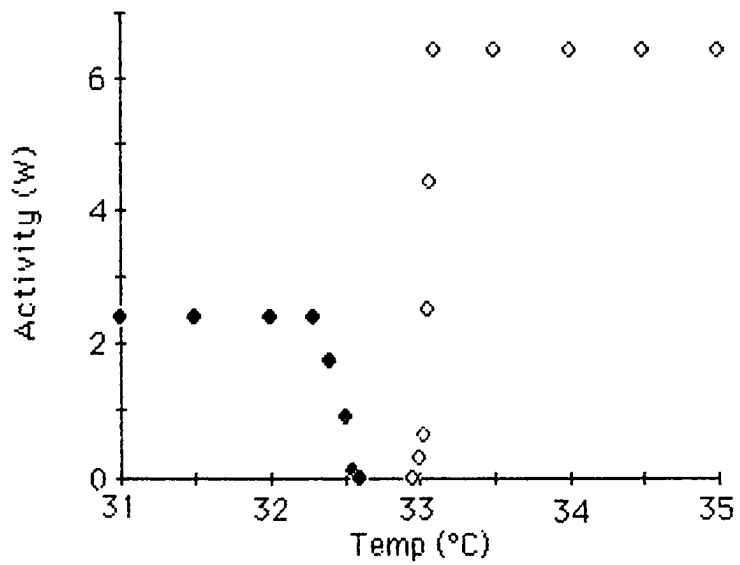

Fig. 1.11. Activity versus temperature profiles of the thermoregulatory effectors for heat loss effector (o) and heat production effector (●).

Fever

Figure 1.12 shows the effect of adding a +2.4 V external input to the Σ stage of the cold sensor to heat production pathway (A in Fig 1.9). The slopes of the activity/temperature curves for both effectors were unaffected, while the intercepts with the abscissa of both curves were shifted upward by 1.0° C. The set point thus shifted upward by 1.0° C to cover the range 33.7° C to 34.0° C. T_{in} was regulated around this new set-point which had the same range as the set-point without any external input.

Applying the same external input to the Σ of the heat sensor to heat loss pathway (C in Fig 1.9) caused the intercepts of the activity/temperature curves to shift in the opposite direction.

Hibernation

Figure 1.13 shows the effect of adding -5.0 V to the Δ stage leading to the heat production effector (B in Fig 1.9). The activity/temperature profile of the cooling device was unaffected. The slope of the activity/temperature profile of the heat production effector was also unaffected, however the intercept with the abscissa was shifted downwards by about 0.8° C. The effect of this shift was to lower the temperature at which the heat production effector became active, and thus shift the lower end of the set-point range from 32.7° C to 31.9° C.

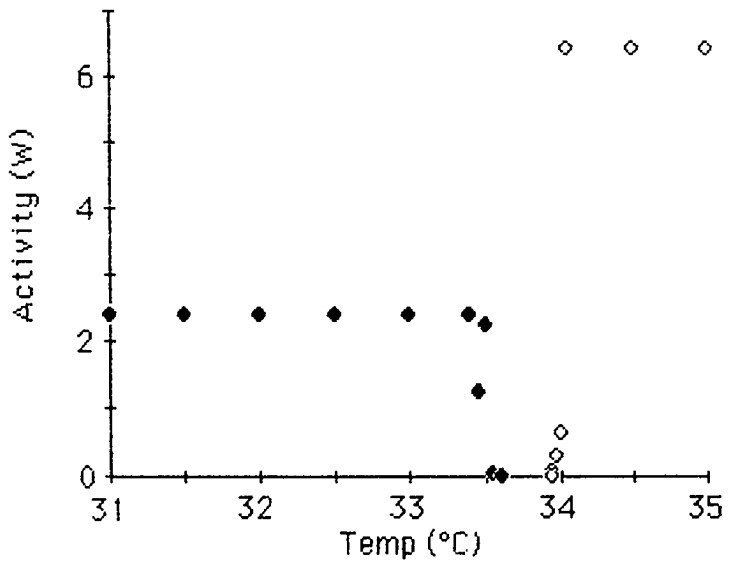

Fig. 1.12. Activity versus temperature profiles of the thermoregulatory effectors after a +2.4 V input was made at point A (Fig 1.9) to simulate the upward shift in set-point during fever. Symbols as in Fig 1.11.

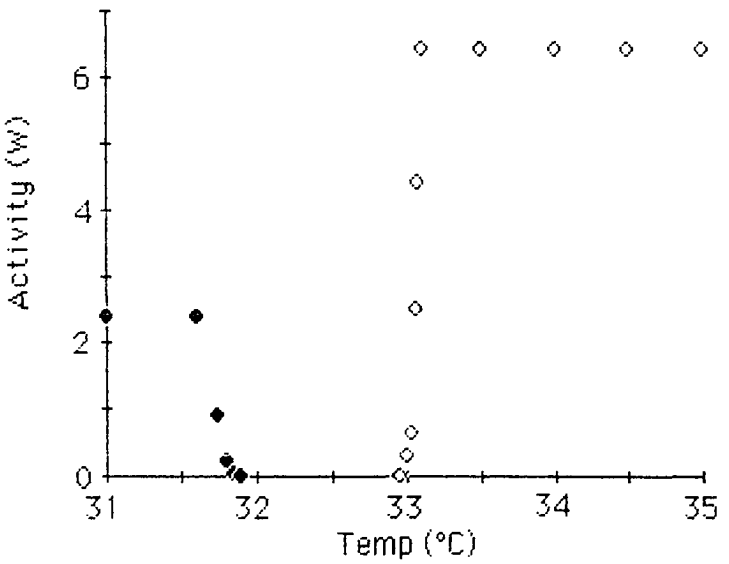

Fig. 1.13. Activity versus temperature profiles of the thermoregulatory effectors after a -5.0 V input was made into point B (Fig 1.9) to simulate the lowering of the heat production threshold during hibernation. Symbols as in Fig 1.11.

Discussion

This model was designed and built in order to demonstrate that thermoregulation can occur without the involvement of a reference signal, and the results have shown this to be the case. The model was not designed as a model of the entire mammalian thermoregulatory system and thus is a very simple system. It did not include any form of peripheral temperature sensors, or peripheral thermal effectors. Whereas it is clear that in the animal there is some form of integration of sensor signals originating from at least the periphery, the spinal cord and the hypothalamus, and that there is a hierarchical recruitment of various forms of effector activity, the final outcome is that temperature is regulated because temperatures are sensed and heat is either produced or lost. Thus, since the main aspect of this study dealt with understanding the neuronal connections in the central nervous system allowing thermoregulation to occur, the details of simulating most of the various mechanisms discussed above were ignored and they were combined into a single cold sensor, a single heat sensor, a single variable heat source and a single cooling device. A few details of mammalian thermoregulation were included in this model, but their inclusion or exclusion were of little consequence to the question at hand. These details included the basal metabolic rate of 2.5 W and the separation of the critical temperatures for the activation of the two thermal effectors creating a null zone rather than a set-point.

It is clear that temperature regulation of the chamber was achieved over a narrow range of ambient temperatures with this model. The

effective range of the model could be extended with the use of more efficient effectors, by increasing the power and gain characteristics of the system or by decreasing the passive heat flow parameters of the chamber. As can be seen from the slopes of the activity/temperature curves of the effectors (Fig 1.11) the gain of the system is currently very high. This high gain was necessary in order to maintain temperature over a 2-3° C gradient. At lower gains T_{in} would drift away from the set-point even with the effectors heating or cooling at full power. This tendency to drift away from the set-point is due to the fact that the only temperature sensors being used in the model are inside the chamber which necessitates a certain amount of drift (dependent on the gain of the system) above or below the set-point before the effectors are activated sufficiently to overcome the heat flow of the chamber. The addition of peripheral temperature sensors with inputs to the Σ stages would allow the system to respond to changes in T_{out} and activate the effectors before T_{in} changes. Thus a tight control could be accomplished without requiring the high gain of this version of the model.

Even though this version of the model was not designed to accurately simulate all aspects of physiological thermoregulation it should be capable of simulating changes in set-point commonly seen *in vivo*, if the neuronal circuitry is accurate. Fever and hibernation are both common conditions in vertebrate thermoregulators in which the set-point is shifted. Fever is characterized by a raised set-point such that after the release of a pyrogen temperature is regulated around a new higher temperature in the same manner as it is regulated in the absence of pyrogen at a lower temperature.

After the disappearance of the pyrogen the temperature returns to the pre-febrile state and is again regulated at the normal level. Based on the results of pharmacological synaptic interference studies Bligh (1979) proposed that pyrogens could be acting by exerting an excitatory effect on the cold sensor to heat production pathway at a point before the origin of the reciprocal inhibitory influence onto the heat loss pathway (A in Fig 1.9). Figure 1.12 shows that when an excitatory input was made at this point in the model the critical temperatures at which heat production and heat loss were activated were both raised, thus raising the set-point. When the excitatory input was removed the set-point and the temperature of the chamber returned to the normal level.

Hibernation and torpor are states characteristic of animals that are normally able to maintain a high and constant body temperature but at certain times apparently abandon homeothermy and allow body temperature to approach environmental temperature. Most hibernators periodically arouse during hibernation bouts and return to homeothermy for brief periods. There also appears to be a lower critical temperature for the activation of heat production such that if core temperature approaches this point arousal may be initiated and core temperature returns to the normothermic level. Thus it seems that thermoregulation is not completely abandoned during hibernation, but rather that the critical temperature for the activation of the heat production pathway is lowered creating a wider null zone describing the set-point. Hammel *et al*(1968) suggested that this could occur through a suppression of the cold sensing neurons of the hypothalamus by an inhibitory signal derived from the ascending reticular activating

system. Bligh (1973) proposed that if this inhibitory signal acted at the Δ stage of the heat production pathway (B in Fig 1.9) the set-point for heat production would be lowered while the heat loss pathway would be unaffected as described above. Figure 1.13 shows that this is exactly what was found when this situation was modeled in the physical system.

The physical model described in this chapter was capable of regulating internal temperature without the involvement of a temperature insensitive reference signal, and furthermore I was able to simulate the changes in set-point seen with the onset of fever and hibernation by including, respectively, excitatory and inhibitory external disturbances in the model. This is not proof that this model describes the actual neuronal pathways of thermoregulation in the central nervous system, but it does show that a reference signal is not required for thermoregulation to occur. It also suggests that this simple model of thermoregulation, which is based on experimentally demonstrated physiological characteristics of thermoregulators, could form the neuronal basis of mammalian thermoregulation.

CHAPTER 2

THE ROLE OF GABA IN A NEURONAL MODEL OF THERMOREGULATION

Introduction

The amino acid gamma-aminobutyric acid (GABA) is widely distributed in cell bodies within the vertebrate CNS, with high concentrations in the hypothalamus. It is most commonly associated with short inhibitory interneurons (Kruk and Pycock 1979), and is considered the major inhibitory neurotransmitter in most regions of the brain. It is involved in at least one-third of all synaptic transmission in the CNS, and between 30 and 45% of all presynaptic terminals in all brain regions studied to date are GABAergic (Johnston 1981). For these reasons alone a possible role for GABA as a synaptically active substance in thermoregulation should be of particular interest to investigators studying the central neurology of thermoregulation.

There have been many studies of the effects of centrally applied GABA, and some of its agonists and modulators, on thermoregulation in a variety of mammals including rats, rabbits, dogs, mice, cats and guinea pigs (Boros-Farkas and Illei-Donhoffer 1969, Sgaragli *et al* 1978, Dhumal *et al* 1974 and 1976, Loscher and Vetter 1984, Schechter and Trainer 1977, Squires 1967 and Komaromi 1976). The results of some of these, and other, studies have been compiled by Clark (1979) and DeFeudis (1984), and show that when GABA is introduced into the ventricles or hypothalamus of this variety of animals it may produce either a rise or fall in core temperature.

Dhumal et al (1976) found that ICV GABA caused hyperthermia at cold and moderate temperatures and prevented this effect at high ambient temperatures in the rat. These results indicate that GABA may have an

overall excitatory role in thermoregulation, however the investigators proposed that these effects may be linked to the effects of prostaglandins. Komáromi et al (1969), Komáromi (1976) and Boros-Farkas and Illei-Donhoffer (1969) found similar excitatory effects on oxygen consumption (VO_2) and body temperature in the neonatal rabbit, neonatal guinea pig and rat respectively, in which both of these variables increased at an ambient temperature slightly below thermoneutral.

Schechter and Trainer (1977) and Löscher and Vetter (1984) pharmacologically increased brain GABA concentrations in the mouse and rabbit respectively, causing hypothermia that was reversible by increasing ambient temperature, thus indicating an inhibitory effect of GABA in thermoregulation. The results of experiments by Sgaragli et al (1978), Squires (1967) and Kerwin and Pycock (1979) in rabbits, cats and rats respectively, indicated some support for this inhibitory role.

The variability in these results could be due to species differences; differences in the point of injection into the ventricular system, or to the structures reached by the injected GABA and in what concentrations; or to the prevailing ambient temperature which would affect the activity along the thermosensor to thermoregulatory pathways and therefore the extent to which such activity could be inhibited. Some papers do not report ambient conditions, and most of the experiments involving the central injection of GABA were done at a single ambient temperature. The only effects that a centrally applied substance can have on core temperature are to raise it, to lower it or to do nothing. Without the observation of concurrent changes in thermoregulatory effector functions, changes observed in core temperature

alone tell us little about the nature of the central disturbance which caused any change in body temperature (Baumann and Bligh 1974). However, the predominating trend of centrally applied GABA appears to be to raise core temperature at high ambient temperatures and to lower it at low ambient temperatures.

Using more rigorous methods including recording the activity of the opposing thermoregulatory effectors (heat production, evaporative heat loss by panting and direct heat loss by changes in peripheral vasomotor tone (PVMT)) as well as core temperature at high, low, and thermoneutral ambient temperautures Bligh *et al*(1979b) found that in sheep intracerebroventricular (ICV) injection of up to 1,000 nmol $\cdot kg^{-1}$ GABA had no effect on the thermoregulatory effectors or on core temperature at any ambient temperature. However the GABA agonist muscimol injected into the ventricles at 0.5-1.0 nmol $\cdot kg^{-1}$ decreased heat production at low T_a ; decreased evaporative heat loss at high T_a and decreased PVMT at ambient conditions near thermoneutrality. They found that another putative central inhibitory transmitter, the amino acid taurine, had similar effects to muscimol when injected into the ventricles. It was suggested that muscimol could be acting at GABAergic receptors, and that the lack of effect of ICV GABA was probably due to its inability to reach these receptor sites. If we accept this reasoning it appears that endogenous GABA is probably an inhibitory transmitter on the major thermoregulatory pathways from warm and cold sensors to heat loss and heat production effectors respectively. Taurine appears to play a similar role in temperature regulation, but it must be born in mind that this substance has not been

accepted as a natural synaptic transmitter in the CNS and could be GABA-mimetic.

The results of an earlier study of the effects of ICV injections of other putative transmitter substances into sheep, goats and rabbits by Bligh *et al* (1971) were described in terms of a model of the central synaptic pathways of thermoregulation (Fig 2.1a). The model consists of two major thermoregulatory pathways; one from warm sensors to heat loss effectors which was activated by ICV 5-hydroxytryptamine (5-HT) which appears to have an excitatory effect, and the other from cold sensors to heat production effectors which was activated by the acetylcholine (ACh) mimetic substance carbachol (CCh) which appears to have an excitatory effect. When the two pathways were activated simultaneously; one by high or low ambient temperature and the other by ICV CCh or 5-HT respectively, the result was not the activation of both pathways but rather the inhibition of the environmentally stimulated pathway (i.e. if an animal was panting in a hot environment an ICV injection of CCh would inhibit panting rather than activate heat production). These findings gave rise to the hypothesis of reciprocal inhibition between the two opposing sensor to effector pathways. Norepinephrine (NE) was found to have an inhibitory effect on whichever pathway was being driven by the ambient temperature as described above for muscimol and taurine, and was assumed to be the terminal transmitter of this reciprocal inhibition.

There is no *a priori* reason to assume that the same transmitter substance is released at the synaptic termination of both of the proposed reciprocal inhibitory pathways, but if it were, the predicted effects of its

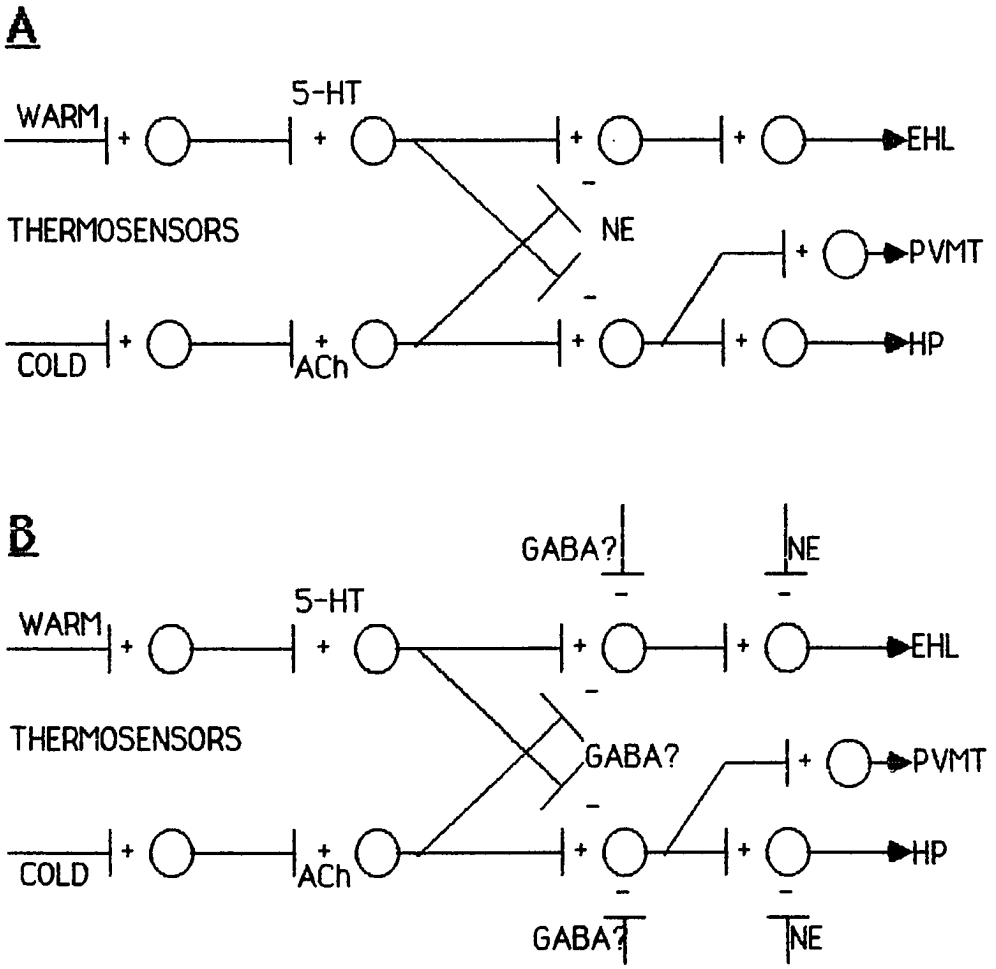

Figure 2.1. The development of neuronal models of the central pathways of thermoregulation by Bligh (1979) interpreting results from injecting synaptically-active substances into the cerebral ventricles of sheep. A. 5-hydroxytryptamine (5-HT) drives evaporative heat loss (EHL) and inhibits heat production (HP), acetylcholine (ACh) drives HP and inhibits EHL, and norepinephrine (NE) inhibits both EHL and HP, giving rise to the hypothesis of NE as the terminal transmitter of reciprocal inhibition. B. NE is a neurotransmitter of converging, not reciprocal, inhibition. The GABA agonist muscimol inhibits all three thermoregulatory pathways and GABA is hypothesized to be a transmitter of converging or reciprocal inhibition, or both. PVMT, peripheral vasomotor tone; +, excitatory signal; -, inhibitory signal.

central application would be those produced by NE, taurine and the GABA agonist muscimol. If NE were the inhibitory transmitter of reciprocal inhibition between the thermoregulatory pathways within the hypothalamus, then NE-containing neurons should be present in the hypothalamus. While NE occurs in nerve fibers in this region of the brain, no NE-containing cell bodies have been found in the region (Dahlstrom and Fuxe 1964), and the NE-containing fibers have been shown to originate in the brain stem (Ungerstedt 1971, Kobayashi *et al* 1974). Further pharmacological studies by Bligh *et al* (1977), Baumann *et al* (1977) and De Roij *et al* (1978) indicated that the evidence for NE as the terminal transmitter of reciprocal inhibition in the sheep was not strong, and it is now proposed that endogenous NE acts as a neurotransmitter of converging inhibition from other brain centers (Fig 2.1b).

If we accept the suggestion discussed earlier that muscimol is acting as a specific GABA agonist in its effects on thermoregulation when injected into the cerebral ventricles, this could indicate a natural inhibitory role for GABA which might relate either to inhibitory fibers converging on the thermosensor to thermoregulatory effector pathways as with NE, or to the hypothesized reciprocal inhibition between the two thermoregulatory pathways (Fig 2.1b). Bligh *et al* (1979c) attempted to sort out these two possibilities by using ICV injections of the GABA antagonist bicuculline-methiodide (Bi) in sheep. They reasoned that if GABA was acting as a natural endogenous inhibitor of thermoregulatory effectors then the administration of a GABA receptor blocker should result in increased heat loss through panting in the hot and increased heat production in the cold. In

preliminary experiments they found that $\dot{V}O_2$ (as a measure of heat production) did increase in the cold after 20 µg ICV Bi, but that the same dose in the hot had no effect on respiratory frequency (Fig 2.2a). They also showed that a prior ICV injection of Bi attenuated the inhibitory effect of ICV CCh on respiratory frequency in the hot, but that it had no effect on the depression of $\dot{V}O_2$ by 5-HT in the cold (Fig 2.2b). These results gave some support to the notion that GABA may function as the terminal transmitter of the hypothesized reciprocal inhibition, but the inconsistencies and preliminary nature of these experiments make them inconclusive. In this chapter I report the results of a series of experiments designed to more rigorously test the hypothesis that GABA acts as the terminal transmitter of reciprocal inhibition between the pathways from cold sensors to heat production effectors and from heat sensors to heat loss effectors.

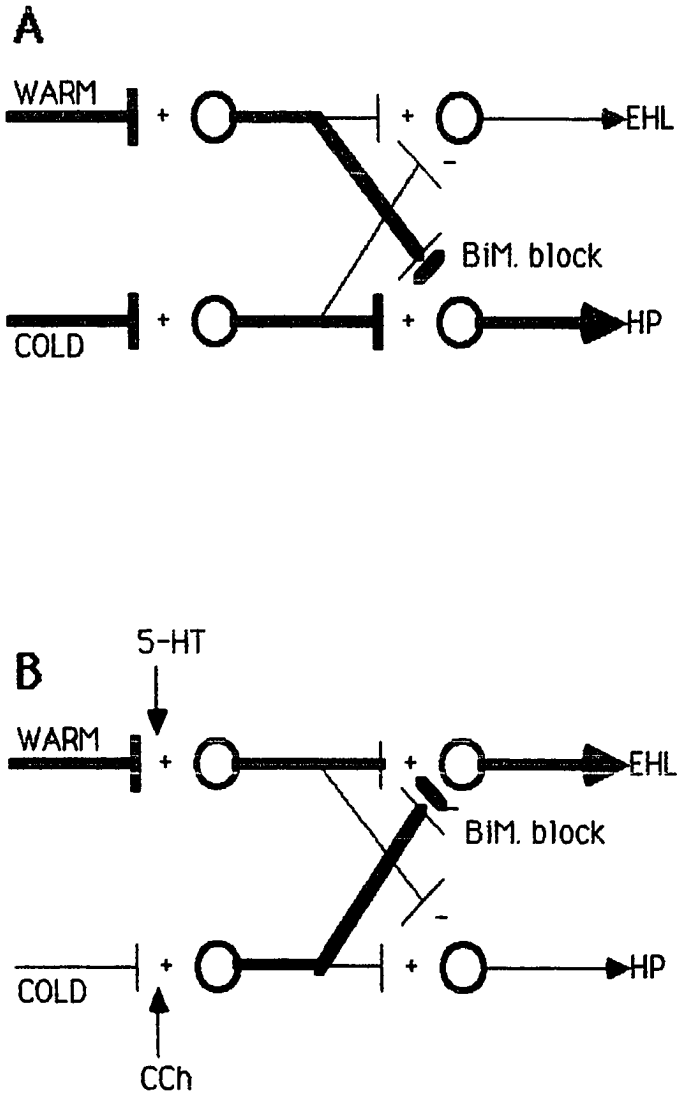

Figure 2.2. Neuronal models depicting the results from ICV injections of the GABA antagonist bicuculline-methiodide (BiM) (Bligh *et al* 1979). A. BiM removes an inhibitory influence on heat production (HP), but not on evaporative heat loss (EHL). B. BiM blocks the attenuating effect of carbachol (CCh) on EHL, but not of 5-HT on HP. Symbols and abbreviations as in Figure 2.1.

Methods

Animals.

Castrated male sheep of mixed breeds between 1 and 3 years old were used in these experiments. The body weights of the animals were maintained between 55 and 70 kg on a diet of water, locally grown hay and barley, plus alfalfa pellets (Burdic Feed, Kent, Washington) and Quality Texture pellets (Fisher Mills, Seattle, Washington). The animals were housed indoors in 1.7m x 2 m pens maintained at 20° C in the Animal Facility of the Institute of Arctic Biology at the University of Alaska, Fairbanks.

All of the animals were subjected to intensive training prior to being used in any experiments to habituate them to human handling and all experimental conditions. At the end of two weeks all animals were quite tractable, they would stand unrestrained on a scale to be weighed and could be led passively into an environmental chamber to be instrumented and left for 1-3 hours while the experiment was in progress.

Cannulation.

At the end of the training period the animals were aseptically cannulated in either the right or left lateral cerebral ventricle under halothane anesthesia. The technique was a modification of that described by Barton *et. al.* (1969) (see Appendix for details) which allowed the placement of the cannula in the main body of the ventricle. The cannula was then kept patent for 3-4 months by flushing with 0.4 ml sterile saline every

two days. In some of the animals a second cannula was placed in the other lateral ventricle when the original was no longer patent, thus doubling the number of possible experiments per animal. A period of at least one week was allowed for recovery under close observation between surgery and the commencement of experiments.

Physiological Measurements.

At least three sheep were used for each treatment, and the animal were rested for at least two days after each experiment involving the injection of synaptically active substances. An animal received no food or water during the experiment and for 24 hours before it. On the day of an experiment one animal was tethered, standing, in a metal stanchion in one of two environmental chambers. One chamber was maintained at 3° C to simulate cold stress on shorn sheep. The other chamber was maintained at 41° C to simulate heat stress on unshorn sheep. Details of ambient temperature and whether or not the sheep were shorn in a particular experiment are given in the Results section.

During an experiment lasting from two to four hours the animals were instrumented to record respiratory frequency (RF), as an indication of evaporative heat loss; oxygen consumption ($\dot{V}O_2$), as an indication of heat production; rectal temperature (T_r), as an indicator of heat storage; and ear skin (T_{ear}) and flank skin temperatures (T_{sk}) as indicators of peripheral vasomotor tone (PVMT). Chamber air, wet and dry bulb temperatures, T_r, T_{sk}, and T_{ear} were measured with copper-constantin thermocouples and recorded every 30 sec on a Leads & Northrup Speedomax Multipoint recorder.

Relative humidity of the chamber was maintained at 35% at 41° C and at about 70% at 3° C as calculated from the wet and dry bulb temperatures using the tables in Consolazio *et al*(1963). The T_r probe was inserted to a depth of 180 mm and secured to the tail by string. The ear and flank thermocouples were held in place by small squares of thin rubber sheeting (100 mm^2) adhered to the skin with a contact adhesive. Respiratory frequency was measured with a pneumograph belt secured around the trunk of the animal and connected to a Gould PM15E differential pressure transducer. Respiratory frequency was recorded for 1 min every 5 min on a polygraph recorder. VO_2 was measured using an open flow system and an Applied Electrochemistry S-3A digital oxygen analyzer, and recorded continuously on a chart recorder. Air was pulled through a head hood at about 60 $l \cdot min^{-1}$ and $\dot{V}O_2$ was calculated using the formula:

$$\dot{V}O_2=(V_{stpd} \cdot (209.3-O_2)) \cdot kg^{-1}$$

$\dot{V}O_2$ = rate of oxygen consumption ($ml \cdot kg^{-1} \cdot min^{-1}$)

V_{STPD} = flowrate of dry outlet air corrected to standard temperature and pressure (l/min)

209.3 = volume concentration of oxygen in inlet air (ml/l)

O_2 = volume fractional concentration of oxygen in outlet air (ml/l) (calculated from record)

kg = body weight of sheep at the beginning of the experiment

An association between an increase in $\dot{V}O_2$ as an index of increased heat production and the occurrence and intensity of shivering was obtained by visually observing the shivering-linked noise of the recording of the respiratory frequency. Other peripheral behavioral and autonomic responses were recorded visually but not quantified.

After being instrumented the sheep was left undisturbed in the chamber, with T_a at either 3 or 41° C, for one hour to record base line information.

After one hour a synaptically active substance was slowly injected into the ventricle through the chronically implanted cannula, and recordings of physiological functions were then continued for two to three hours. All drugs were injected in 0.2 ml sterile pyrogen-free 0.9% saline followed immediately by another 0.2 ml saline. When saline was given as a control injection the total volume of 0.4 ml was given as a single injection. In some experiments more than one substance was injected. In these cases the first drug was always given 20 min prior to the second drug. Each solution was passed through a 0.22 µm GS Millipore filter before injection. The drugs used were gamma-aminobutyric acid (GABA), the GABA agonist muscimol (3-hydroxy-5-aminomethylisoxalole), the GABA antagonists bicuculline-methiodide (Bi) and picrotoxin, the acetylcholine agonist carbamylcholine (CCh) and 5-hydroxytryptamine (5HT). All drugs were obtained from Sigma Chemical Co., St. Louis, Missouri.

Drug doses are expressed as moles of their salts. In previous reports from this lab doses have been expressed in terms of moles per unit body weight. I originally became concerned about this method of calculating

doses because the animals used in these experiments were about twice as heavy as the Welsh Mountain sheep used in the earlier work. This meant that a given weight specific dose actually contained about twice as many molecules of a synaptically active substance being injected into the ventricles in my experiments than in the earlier work, and that within my own experiments the dose increased by 50% from the smallest to the largest animals. The weight differences were almost totally in body fat as estimated by visual estimates of live animals. Visual observations of brains of various sized sheep showed little difference in brain or ventricular volume. My initial concern became justified when two animals received overdoses of Bi given on a weight specific dose. From that point on all doses were calculated on a total molecular basis, and there were no further problems with overdoses. Although this method better relates dose to brain size, there was still wide variability between animals in their responses to a given dose of a drug. The variability could be due to variations in the placement of the cannula within a lateral ventricle or to variations in pressure and/or flow rate within the ventricle or various other possibilities. This variability was not a problem in my experiments as the responses, although quantitatively variable, were not qualitatively variable between animals receiving the same treatment. One animal was given a lower dose of Bi (33 instead of 39 nmol) than that given to the other two animals in the final set of experiments because of its greater responsiveness to the higher dose. The doses of all substances used in these experiments, except GABA, were based on dose responses determined in previous work from this laboratory and found satisfactory for our

experiments. Since GABA had not previously been shown to have any effect on thermoregulation in sheep at any dose, a dose response curve was determined by intracerebroventricular (ICV) injections at 25° C. A slight short-term depression of RF was seen in doses down to 5.5 µmol and was graded up to the highest dose used (39µmol).

Results are presented as mean ± standard error, and statistical comparisons are based on Student's t-distribution tests. A change in T_r was considered significant if 60 min after an ICV injection the mean T_r of all animals receiving the same injection differed from the mean values recorded at 5 min intervals for 30 min prior to the injection at $P \leq 0.05$. RF and $\dot{V}O_2$ were compared as means of the responses recorded at 5 min intervals for 30 min periods following all ICV injections against the mean of the values recorded at 5 min intervals for the 30 min control period preceding any injections, unless stated otherwise in the Results section. These differences were considered statistically significant if $P \leq 0.05$. Physiologically significant changes will not be determined by any *a priori* definitions, and will be discussed individually.

Results

Peripheral vasomotor tone

At extreme temperatures peripheral vasomotor tone (PVMT), as determined from ear skin and flank skin temperatures, does not usually respond to ICV injections of drugs (Bligh *et al* 1979a). At high T_a the peripheral blood vessels remain dilated and at low T_a they remain constricted. Apparently at extreme temperatures the effects exerted by the local temperature sensed by the skin receptors normally overrides any central influence. Thus, since all of my experiments were done at either high or low T_a it is not surprising that I found no significant change in PVMT in response to any of the substances administered. This is not meant to imply that these substances have no effect on PVMT, and had the experiments been performed under less stressful thermal conditions some responses may have been seen .

Effects of ICV injections of 0.9% sterile saline in shorn sheep at 3° C and in unshorn sheep at 41° C.

For statistical analysis each experiment acted as its own control with the 30 min period preceding the initial ICV injection being the control period. In order to verify the claims by Bligh *et al* (1971) that ICV saline was without effect on thermoregulation, and to provide a further control for my experiments I performed this set of control experiments.

In unshorn sheep at 41° C ICV saline had no effect on RF or $\dot{V}O_2$ (Fig 2.3, Table 2.1). Although the effector activities had stabilized at levels

Table 2.1. Thermoregulatory effects of intracerebroventricular injections of various drugs in unshorn sheep at 41° C ambient temperature. Changes in RF and VO_2 are the differences of the means of the responses recorded at 5 min intervals for 30 min before and after all injections, unless indicated otherwise. Results are presented ± standard error. NS, no significant change; +, increase; −, decrease.

			RF			VO_2		
Drug	Dose	No. of Expts.	Change in RF (breaths·min^{-1})	% Change	Significance	Change in VO_2 (mlO_2·kg^{-1}·min^{-1})	% Change	Significance
NaCl		3	+10 ± 8*	+3.0	NS	+0.56 ± 0.43*	+10.0	NS
GABA	39µmol	5	-94 ± 13	-40.0	(P<0.001)	-0.19 ± 0.14	-5.0	NS
Muscimol (Mu)	35nmol	3	-146 ± 21	-58.0	(P<0.001)	+0.07 ± 0.28	+1.0	NS
Bicuculline Meth.(Bi)	39nmol	5	+68 ± 13**	+29.0	(P<0.001)	+2.75 ± 0.76	+68.0	(P<0.001)
Bi		5	-69 ± 30***	-30.0	(P<0.05)			
Bi/Mu	39/35nmol	4	+57 ± 18	+25.0	(P<0.01)	+0.95 ± 0.25	+22.0	(P<0.001)
Bi/Mu		4	-6 ± 14****	-3.0	NS	+0.28 ± 0.24****	+7.0	NS
Bi/GABA	39nmol/39µmol	2*****	+72 ± 45	+30.0	NS	+2.44 ± 0.27	+65.0	(P<0.001)
CCh	137nmol	3	-212 ± 14	-80.0	(P<0.001)	-0.31 ± 0.37	-6.0	NS
Bi/CCh	39nmol/137nmol	5	-24 ± 17	-12.0	NS	+2.88 ± 0.65	+64.0	(P<0.001)

* Control periods for RF and VO_2 are the 15 min periods preceding injection.
** Change in RF for the 30 min period from 40-70 min after injection.
**** Change in RF 5 min after injection.
**** Change in RF and VO_2 for the 30 min period from 30-60 min after both injections.
***** This experiment was done two times on a single animal.

Table 2.1

Table 2.2. Thermoregulatory effects of intracerebroventricular injections of various drugs in shorn sheep at 3° C ambient temperature. Changes in RF and VO_2 are the differences of the means of the responses recorded at 5 min intervals for 30 min before and after all injections, unless indicated otherwise. Results are presented ± standard error. NS, no significant change; +, increase; -, decrease.

			RF			VO_2		
Drug	Dose	No. of Expts.	Change in RF (breaths·min^{-1})	% Change	Significance	Change in VO2 ($mlO_2 \cdot kg^{-1} \cdot min^{-1}$)	% Change	Significance
NaCl		3	+1 ± 1	+10.0	NS	+1.03 ± 0.27	+12.0	(P<0.001)
GABA	39µmol	3	-1 ± 1	-5.0	NS	-2.64 ± 0.68	-27.0	(P<0.001)
Muscimol (Mu)	35nmol	3	-1 ± 1	-4.0	NS	-2.74 ± 0.57	-32.0	(P<0.001)
Bicuculline Meth.(Bi)	39nmol	3	+7 ± 2	+45.0	(P<0.001)	+5.36 ± 1.04	+64.0	(P<0.001)
Bi/Mu	39/35nmol	3	+11 ± 3	+59.0	(P<0.01)	+0.90 ± 0.90	+10.0	NS
Bi/Mu		3	-1 ± 1*	-5.0	NS	-2.60 ± 0.54*	-29.0	(P<0.001)
5-HT	1.6µmol	3	-1 ± 1	-3.0	NS	-1.53 ± 0.36	-17.0	(P<0.001)
Bi/5-HT	39nmol/1.6µmol	4	+11 ± 2**	+67.0	(P<0.001)	+1.59 ± 0.95	+19.0	NS
Bi/5-HT		4	+99 ± 35***	+482.0	(P<0.01)			

* Change in RF and VO_2 for the 30 min period from 30-60 min after both injections.
** Change in RF for the 25 min period after both injections.
*** Change in RF for the 15 min period between 25 and 40 min after both injections.

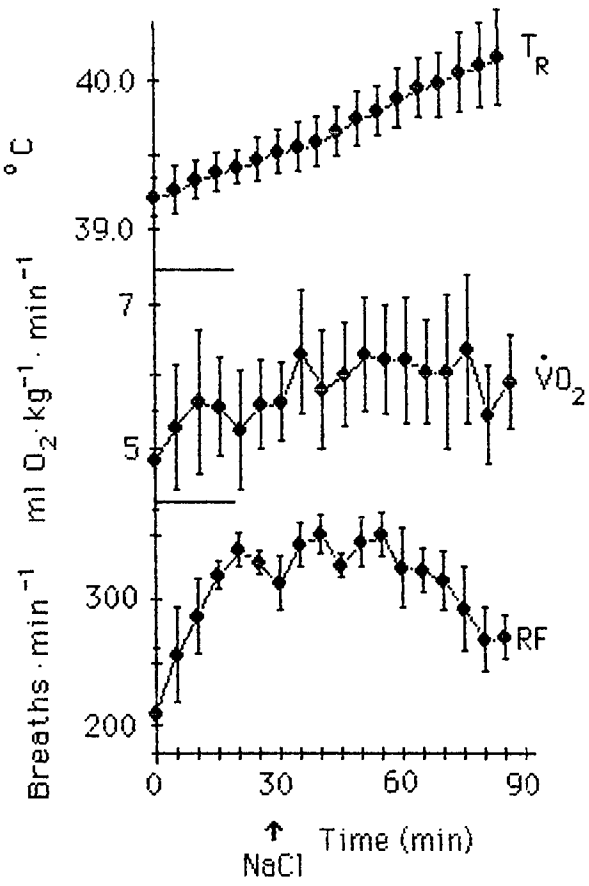

Figure 2.3. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of saline. Ambient temperature 41° C; unshorn sheep; N = 3. T_R, rectal temperature: $\dot{V}O_2$, oxygen consumption; RF, respiratory frequency.

appropriate for the ambient temperature at the time of the injection, T_r was not stable and continued to increase at the same rate after the injection.

In shorn sheep at 3° C ICV saline did not affect T_r or RF, but it did cause $\dot{V}O_2$ to increase by 12% ($P<0.001$) for 30 min (Fig 2.4, Table 2.2).

Effects of ICV injections of GABA, muscimol and Bi at 3 and 41 °C.

GABA

The ICV injection of 39 µmol GABA inhibits both major thermoregulatory pathways for about 30 min. In unshorn sheep at high T_a this dose caused a 40% decrease in RF ($P<0.001$) and an increase in T_r while not affecting $\dot{V}O_2$ (Fig 2.5, Table 2.1). In shorn sheep at low T_a it caused a 27% decrease in $\dot{V}O2$ ($P<0.001$) and no effect on RF, which was already maximally depressed, or T_r (Fig 2.6, Table 2.2). These effects are summarized in Table 2.1.

Muscimol

The ICV injection of 35 nmol of the GABA agonist muscimol decreased RF by 58% ($P<0.001$) and increased T_r at high T_a in unshorn sheep (Fig 2.7, Table 2.1). In shorn sheep at low T_a it decreased $\dot{V}O_2$ by 32% ($P<0.001$)(Fig 2. 8, Table 2.2). This dose was less than 1/1,000 the dose of GABA discussed above and the effects lasted for about 90 min.

A number of other nonthermoregulatory reactions to GABA and muscimol were noted but not quantified. At high T_a ICV muscimol increased feeding behavior as identified by the increase in chewing motions and

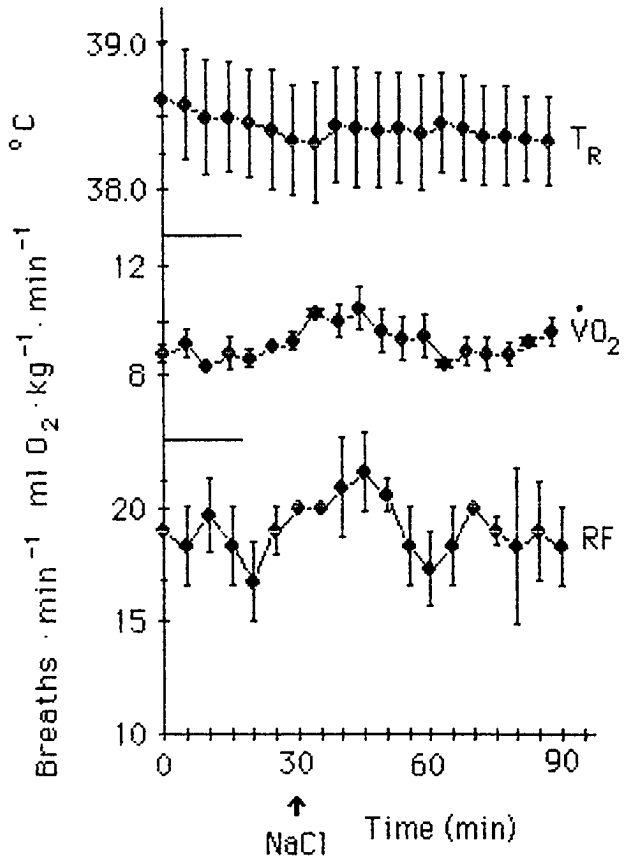

Figure 2.4. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of saline. Ambient temperature 3° C; shorn sheep; N = 3. Symbols as in Figure 2.3.

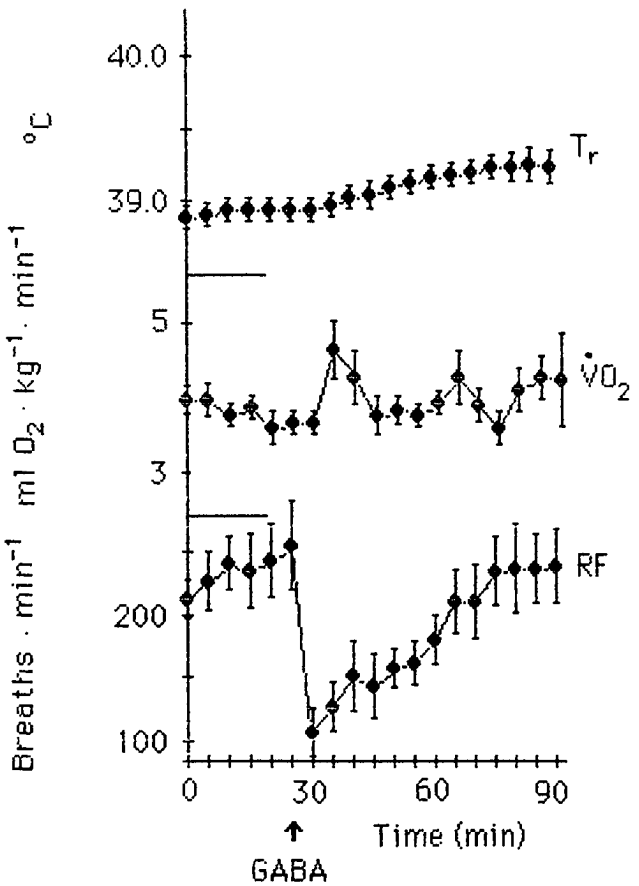

Figure 2.5. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of 39 µmol GABA. Ambient temperature 41° C; unshorn sheep; N = 3. Symbols as in Figure 2.3.

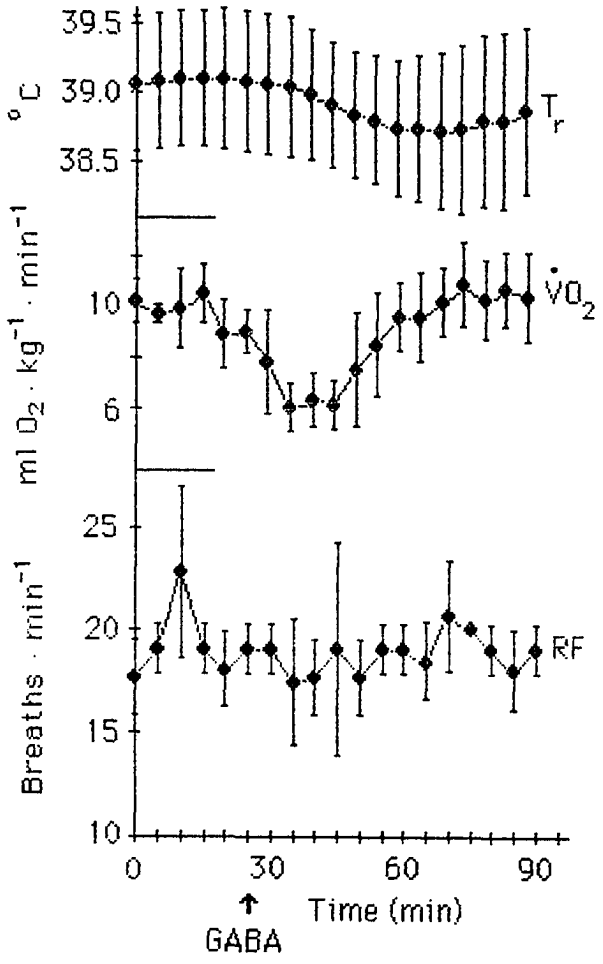

Figure 2.6. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of 39 µmol GABA. Ambient temperature 3° C; shorn sheep; N = 3. Symbols as in Figure 2.3.

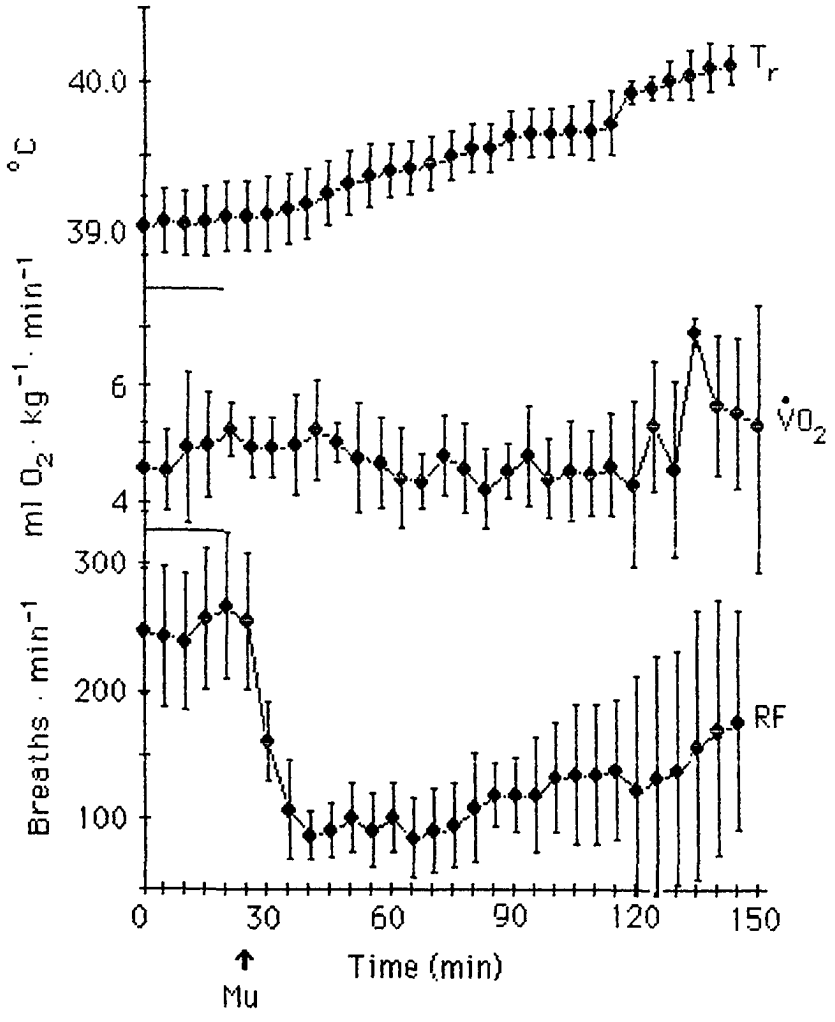

Figure 2.7. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of 35 nmol muscimol. Ambient temperature 41° C; unshorn sheep; N = 3. Symbols as in Figure 2.3.

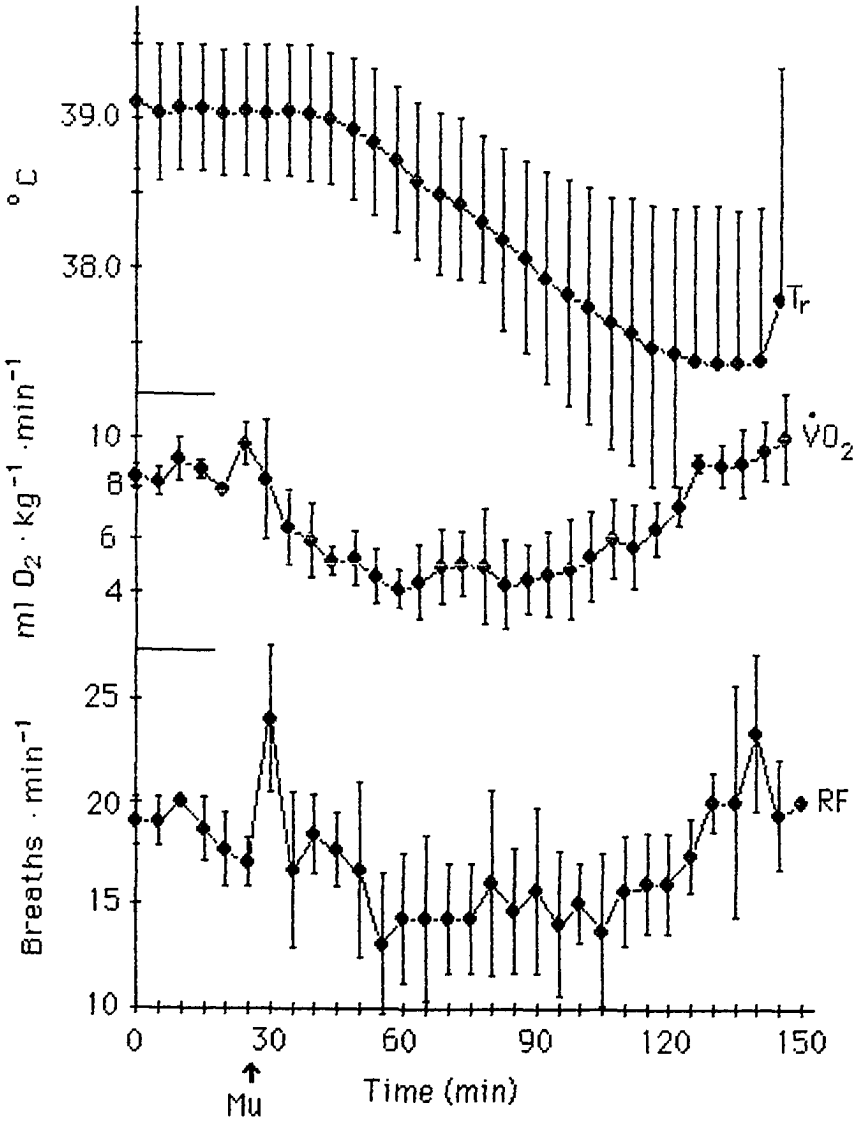

Figure 2.8. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of 35 nmol muscimol. Ambient temperature 3° C; shorn sheep; N = 3. Symbols as in Figure 2.3.

licking of the inside of the metabolism hood following the injection. A similar, but reduced, response was also seen after ICV GABA at high T_a. This is in partial agreement with the work of Seoane *et al*(1984) in which they report a large increase in food intake of sheep after ICV muscimol, but no effect after ICV GABA. At high T_a ICV muscimol also caused a large increase in urination, in agreement with results of intrahypothalamic injections of muscimol in mice (Kelly *et al* 1979). At low T_a urine output is already high, and no change was noted after administration of muscimol. Muscimol, and sometimes GABA, caused a decrease in activity as the increased feeding response declined in the heat and the cold, with two animals apparently sleeping for short periods. This decline in activity always appeared after the onset of the thermoregulatory responses discussed above.

Bicuculline-methiodide and Picrotoxin

The ICV injection of 39 nmol of the GABA antagonist Bi had an overall excitatory effect on both heat production and heat loss in the cold and the heat (Tables 2.1 and 2.2). The response of the RF of the unshorn sheep at 41° C was bimodal (Fig 2.9). There was an initial 30% drop ($P<0.05$) lasting about 5 min, followed by a rise to about 30% above the control value ($P<0.001$) for over 2 h. $\dot{V}O_2$ rose to double the control value for a short period and then remained about 35% above the control value ($P<0.001$) for over 2 h (Fig 2.9). The initial rise in $\dot{V}O_2$ was sometimes accompanied by an onset of shivering for a short period. The RF of the shorn sheep at 3° C rose by 45% ($P<0.001$) for 30 min and the $\dot{V}O_2$ again doubled ($P<0.001$) for a short

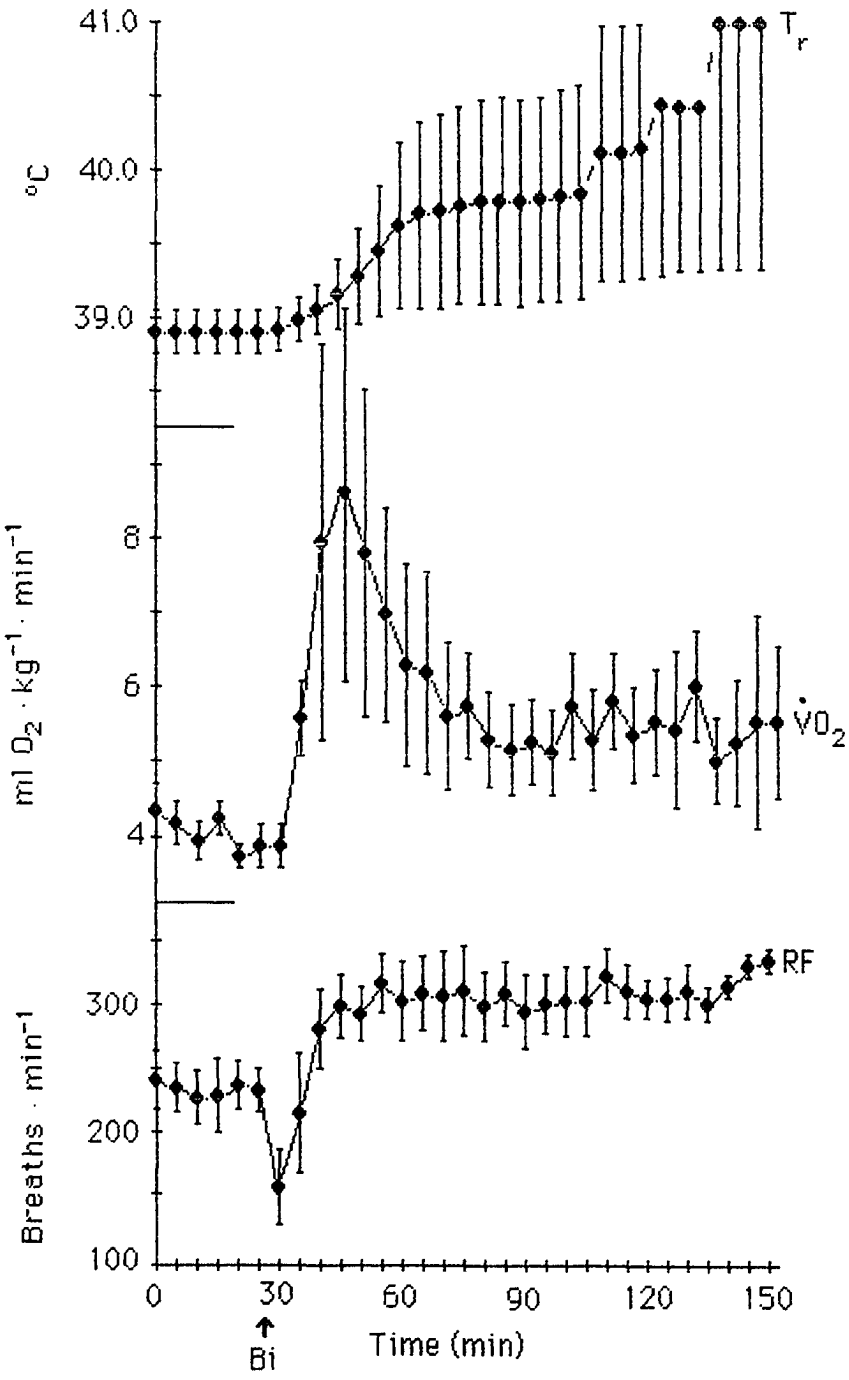

Figure 2.9. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of 39 nmol bicuculline-methiodide. Ambient temperature 41° C; unshorn sheep; N = 5. Symbols as in Figure 2.3.

period, returning to the control value after 30 min (Fig 2.10). This increase in $\dot{V}O_2$ was always accompanied by an increase in shivering. At 3° C T_r increased after ICV Bi.

Previous work in this laboratory had shown that 30 nmol of the GABA antagonist picrotoxin had essentially the same effect as 39 nmol Bi in the cold and heat. I performed a single experiment on one animal with this dose of picrotoxin in the cold. The results were similar to those of the Bi experiments (Fig 2.11). I felt that this verified the assumption that these two substances were both acting as GABA-blockers and decided to use only Bi in the remainder of the experiments requiring a GABA-blocker.

The injections of both Bi and picrotoxin caused increases in activity of the animals. This was identified by an onset of bleating, pawing of the ground and lip-licking.

Effects of prior ICV injections of Bi on the effects of ICV injections of muscimol and GABA at 3 and 41° C.

The ICV injection of 39 nmol Bi 20 min prior to an ICV injection of either 39 µmol GABA or 35 nmol muscimol into an unshorn sheep at 41° C removed the depression of RF normally caused by these doses of GABA and muscimol at this temperature (Table 2.1). At 3° C this experiment was performed with muscimol on shorn sheep and it was found that the bicuculline blocked the depression of $\dot{V}O_2$, normally caused by 35 nmol muscimol, for 30 min after the muscimol injection (Table 2.2).

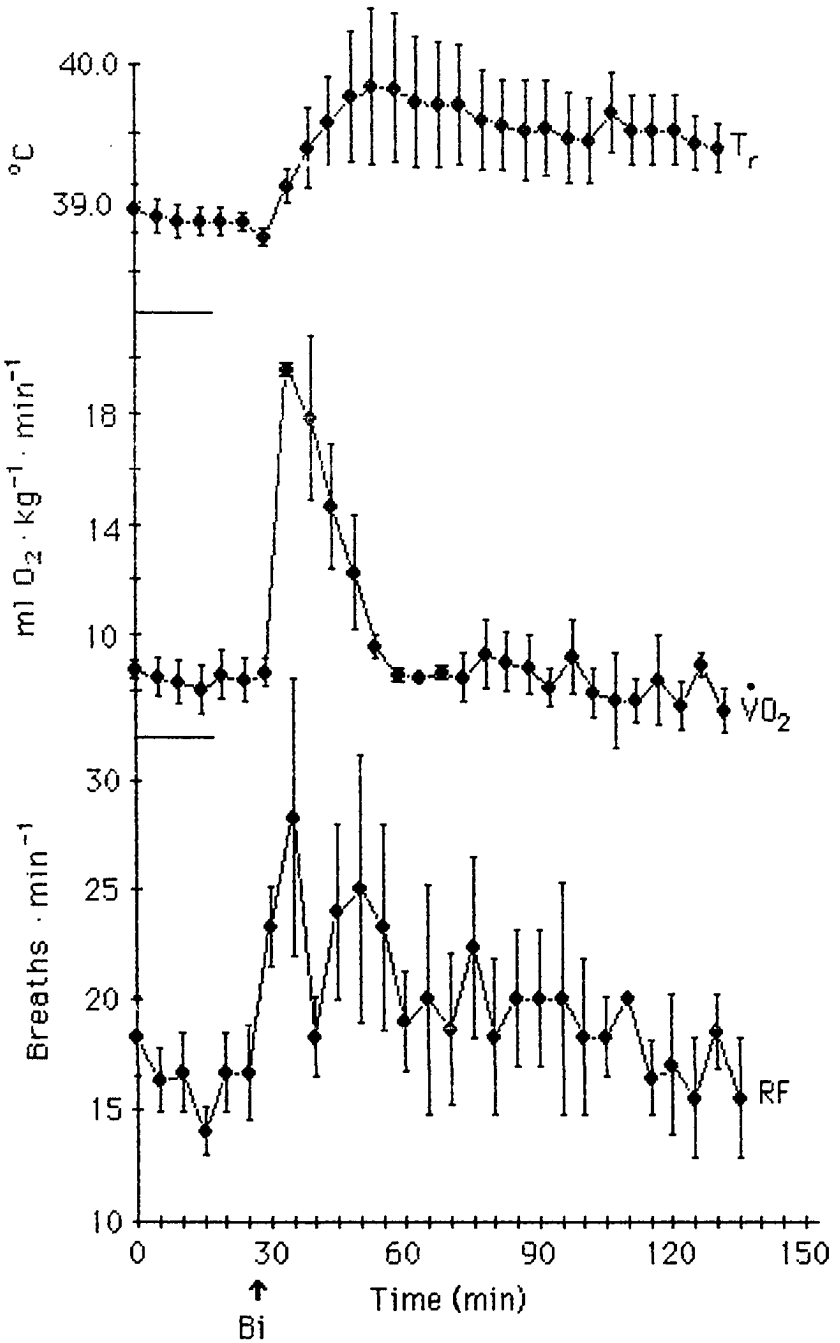

Figure 2.10. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of 39 nmol bicuculline-methiodide. Ambient temperature 3° C; shorn sheep; N = 3. Symbols as in Figure 2.3.

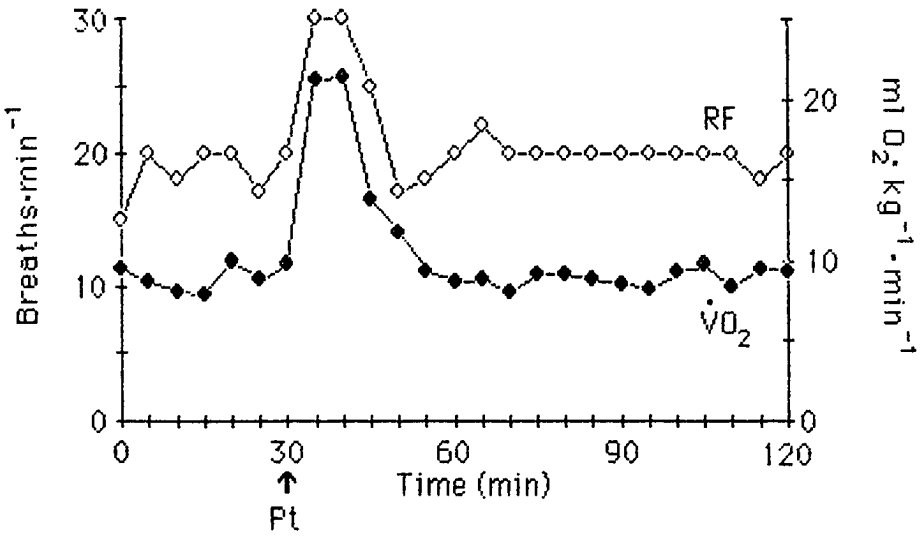

Figure 2.11. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of 30 nmol picrotoxin. Ambient temperature 3° C; shorn sheep; N = 1. $\dot{V}O_2$, oxygen consumption; RF, respiratory frequency.

Effects of ICV injections of 137 nmol Carbachol at 41° C in unshorn sheep and 1.6 µmol 5-hydroxytryptamine at 3° C in shorn sheep.

These experiments have been done and reported previously by Bligh *et al*(1971). My results were in agreement with those previously reported for both sets of experiments. 137 nmol CCh in the unshorn sheep at 41° C decreased RF by about 80% ($P<0.001$), did not significantly increase $\dot{V}O_2$ and increased T_r (Fig 2.12, Table 2.1). In the shorn sheep at 3° C 1.6 µmol 5-HT caused about a 20% decrease in $\dot{V}O_2$ ($P<0.001$), no significant change in RF and a fall in T_r (Fig 2.13, Table 2.2).

Effects of ICV injections of 39 nmol Bi on the response of unshorn sheep to 137 nmol CCh at 41° C and shorn sheep to 1.6 µmol 5-HT at 3° C.

In the previous set of experiments both thermoregulatory pathways were stimulated, one by ambient temperature and the other by an ICV injection of either CCh (heat production) (Fig 2.14a) or 5-HT (heat loss) (Fig 2.14b). As discussed above, in this experiment the pathway stimulated by the ICV injection of CCh or 5-HT did not become active while the environmentally stimulated pathway was inhibited. Since my work thus far was beginning to implicate GABA as the inhibitory agent which could relate to reciprocal inhibition I attempted this same experiment but with an ICV injection of the GABA antagonist Bi before the ICV injections of CCh or 5-HT to see if both heat production and heat loss effectors would become active under these conditions.

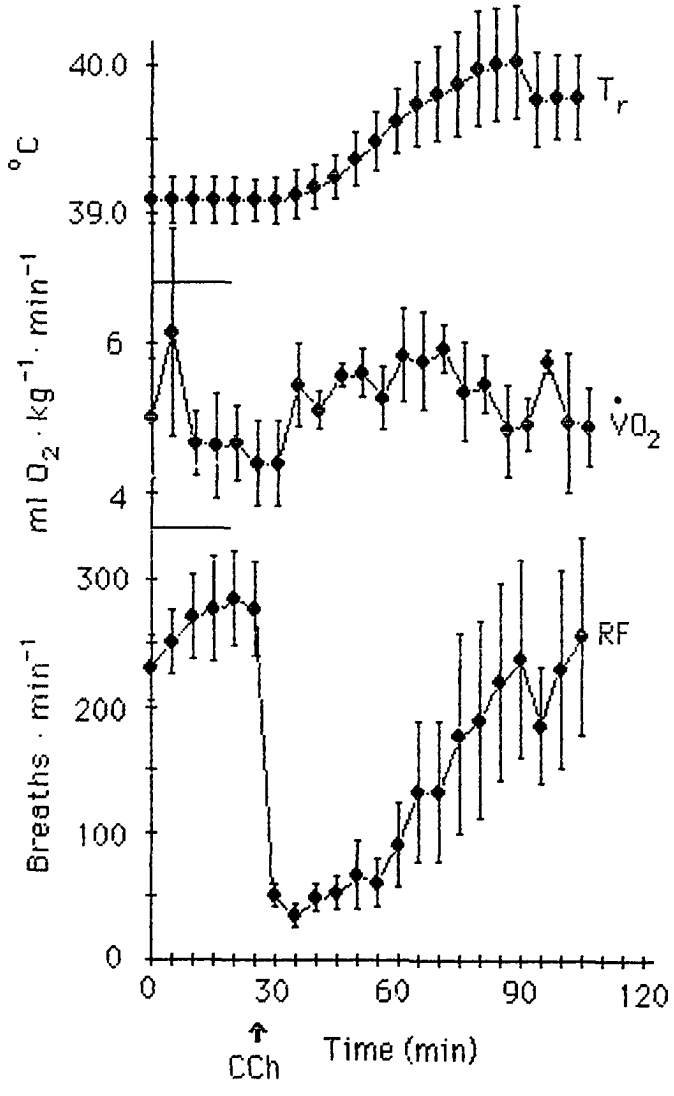

Figure 2.12. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of 137 nmol carbachol. Ambient temperature 41° C; unshorn sheep; N = 3. Symbols as in Figure 2.3.

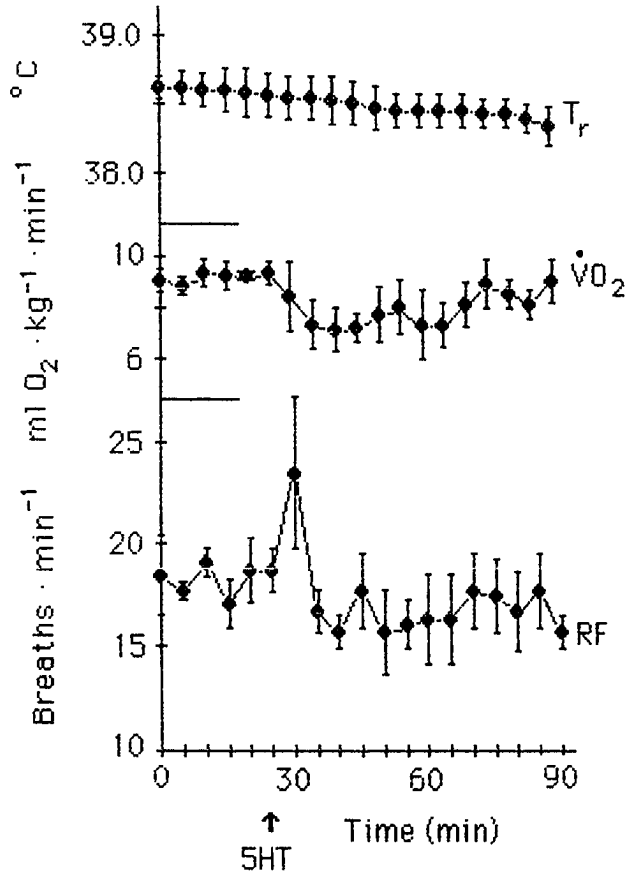

Figure 2.13. The effects on thermoregulatory effectors produced by the ICV injection (arrow) of 1.6 µmol 5-hydroxytryptamine. Ambient temperature 3° C; shorn sheep; N = 3. Symbols as in Figure 2.3.

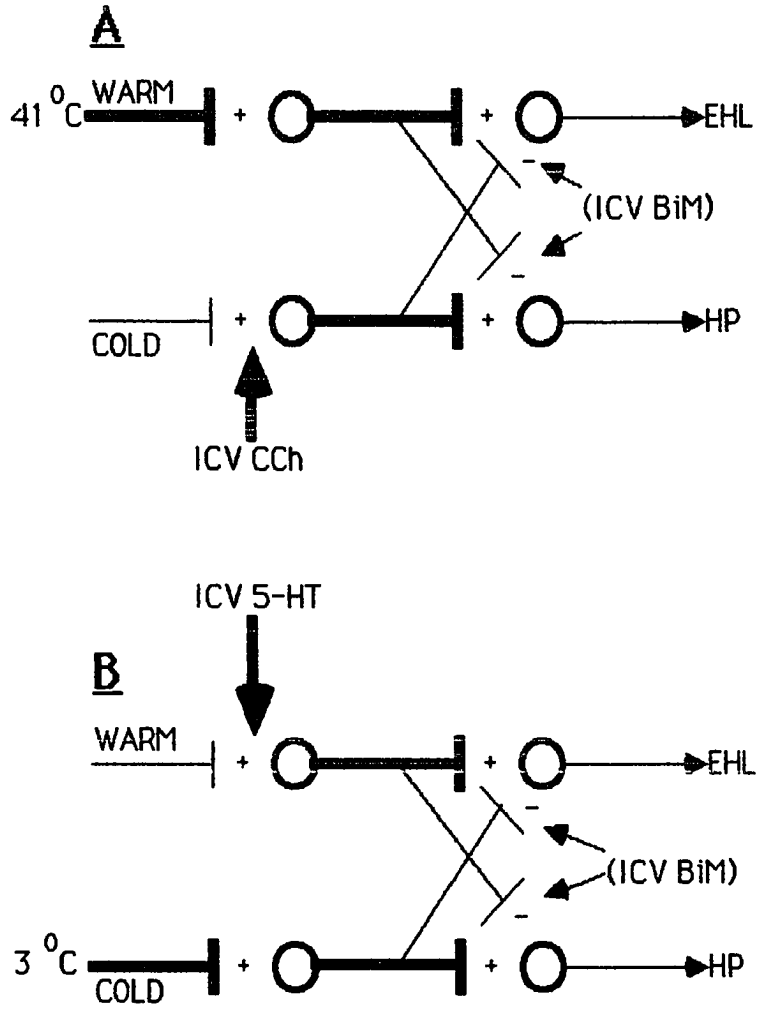

Figure 2.14. Representations of experiments in which both heat production and heat loss are stimulated. A. Evaporative heat loss (EHL) stimulated by high ambient temperature (41° C) and heat production (HP) by ICV injection of CCh. B. HP stimulated by low ambient temperature (3° C) and EHL by ICV injection of 5-HT. BiM., bicuculline-methiodide (a GABA antagonist).

When inhibition by GABA was blocked by a prior ICV injection of Bi the normal inhibition of RF by CCh (Fig 2.12) in the heat was not seen while $\dot{V}O_2$ was elevated above the control value by about 60% ($P<0.001$) for 60 min after ICV CCh (Fig 2.15, Table 2.1). In this case the CCh does cause RF to return to the control level rather than remaining 30% elevated as seen with Bi alone (Fig 2.9), but this does not approach the 80% decrease seen with CCh alone (Fig 2.12).

Pretreatment with an ICV injection of Bi in the shorn sheep in the cold delayed the usual 5-HT mediated inhibition of $\dot{V}O_2$ (Fig 2.13) by 30 min (Fig 2.16). Respiratory frequency increased by 67% ($P<0.001$) for the 25 min following ICV 5-HT and approximately thirty minutes after the 5-HT injection RF rose from about 28 breaths $\cdot$ min $^{-1}$ to over 100 breaths $\cdot$ min $^{-1}$ ($P<0.01$) for about 15 min (Table 2.2). RF of one animal reached 400 breaths $\cdot$ min $^{-1}$ during this period.

In both cases I was able to activate heat production and heat loss by blocking GABA. At high T_a both pathways were active simultaneously as expected, but for some reason at low T_a the heat loss pathway did not become fully active until after heat production had returned to the control level.

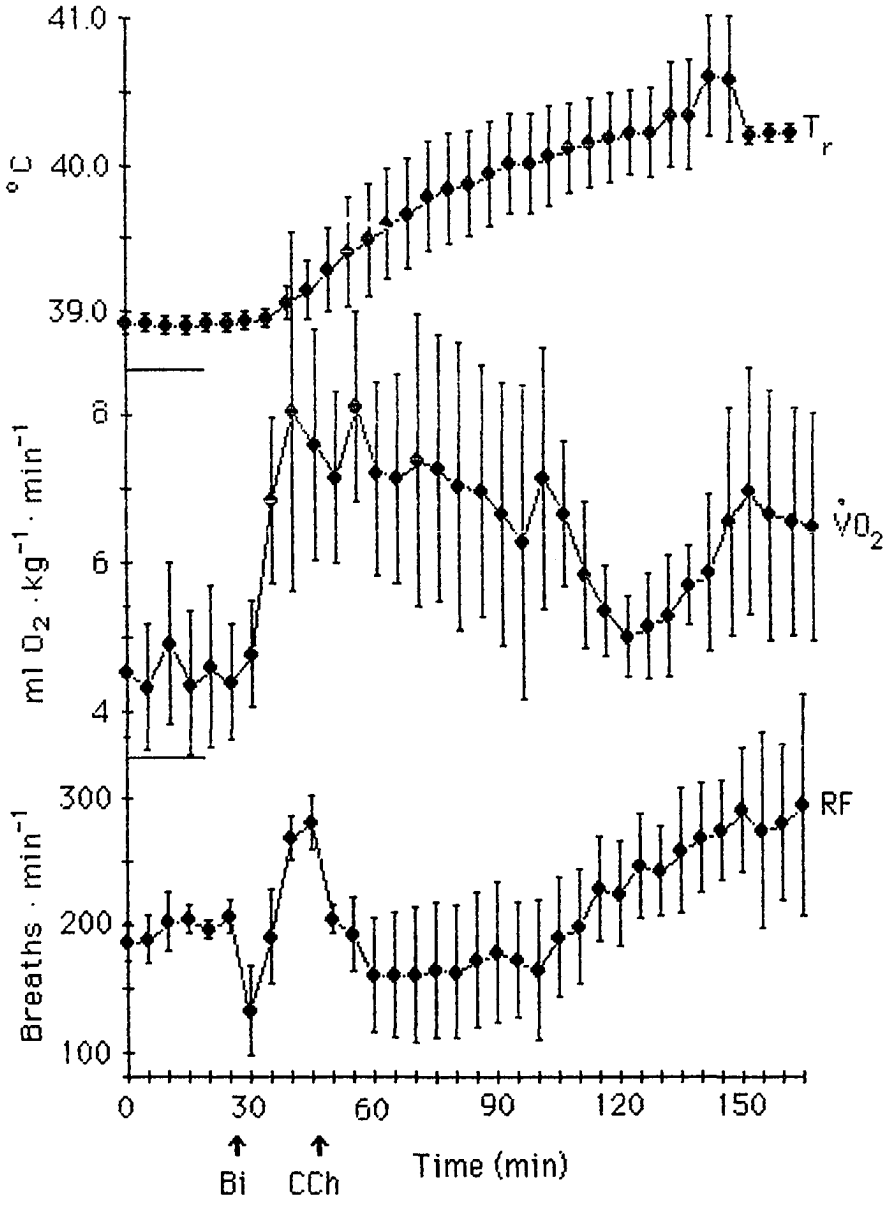

Figure 2.15. The effects on thermoregulatory effectors produced by the ICV injection (first arrow) of 39 nmol bicuculline-methiodide followed 20 min later (second arrow) by the ICV injection of 137 nmol carbachol. Ambient temperature 41° C; unshorn sheep; N = 5. Symbols as in Figure 2.3.

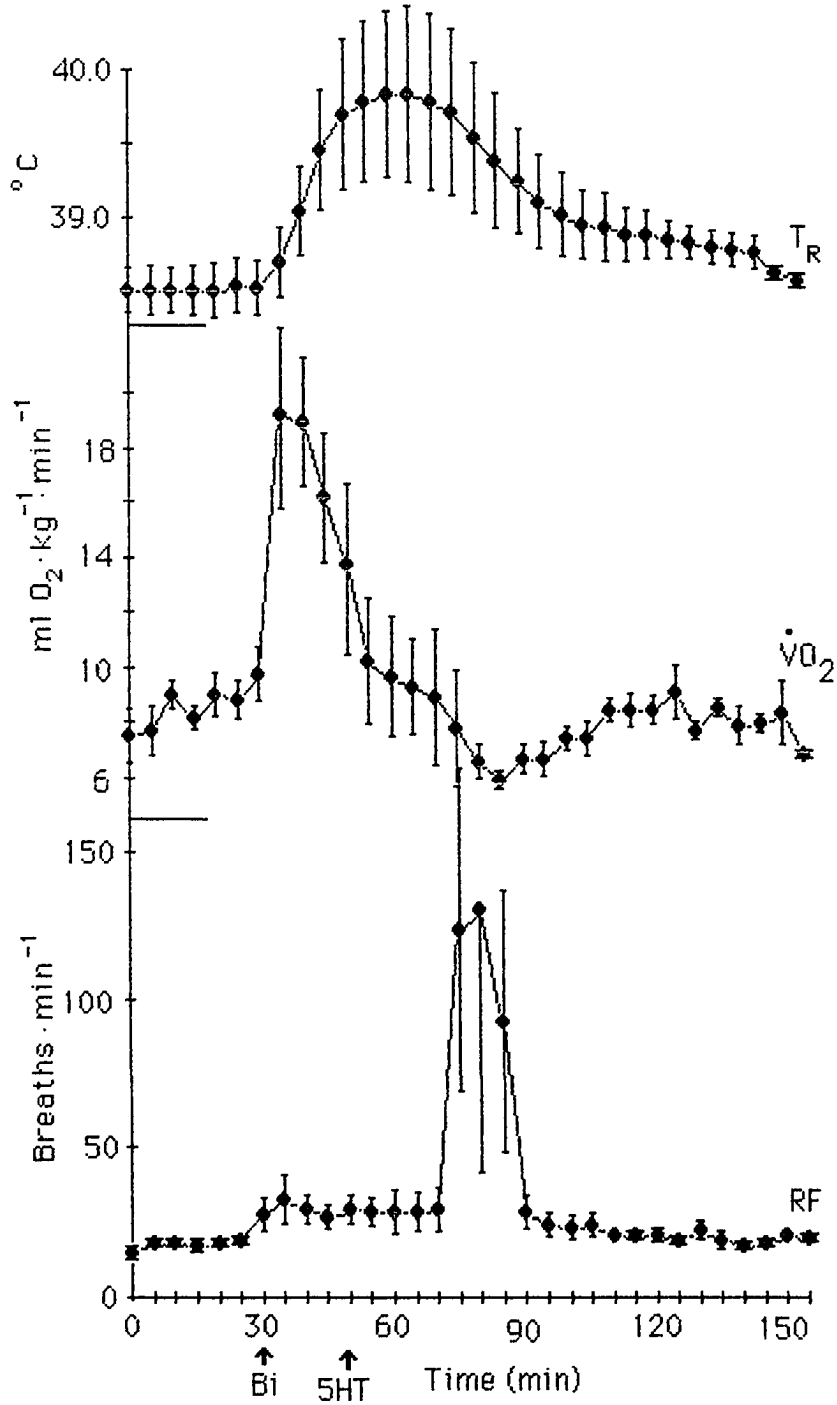

Figure 2.16. The effects on thermoregulatory effectors produced by the ICV injection (first arrow) of 39 nmol bicuculline-methiodide followed 20 min later (second arrow) by the ICV injection of 1.6 µmol 5-HT. Ambient temperature 3° C; shorn sheep; N = 4. Symbols as in Figure 2.3.

Discussion

Neuroactive substances injected into the cerebral ventricles can influence a wide variety of physiological functions and produce simultaneous and inappropriate excitation and/or inhibition of many different neuronal pathways. These responses to unnatural aggravation of brain activities could give results unrelated to the normal patterns of response and might mask or enhance the particular responses of interest. In light of these cautions the use of intracerebroventricular (ICV) injections in this study must be justified and the interpretation of the results must be made with these cautions in mind.

If ICV injections of synaptically-active substances did cause massive interference of populations of neurons relating to physiological functions other than thermoregulation it might be expected that a mass of uncoordinated changes in effector functions would be elicited, and that these would be random and defy interpretation. In fact, Bligh (1981) has clearly demonstrated that ICV injections of many synaptically-active substances produce clear and repeatable effects on thermoregulation that have been readily interpretable in terms of a simple neuronal model (Fig 2.1b).

It has been suggested that injections directly into the areas of the brain involved in thermoregulation would produce more meaningful results than ICV injections. There is no doubt that this method may prove useful and that continuation of this work will require it. However, although there is good reason to assume that the preoptic/anterior hypothalamic region of

the lower forebrain is the principal area of integration of the thermoregulatory pathways there is also good reason to believe that these pathways are multisynaptic and that not all of the synapses necessarily lie within the preoptic/anterior hypothalamus. Because of this, coupled with the highly localized nature of intrahypothalamic injections, negative results obtained with this method might be meaningless. The only realistic interpretation of a negative result is that possibly not all of the receptors necessary to produce a thermoregulatory effect were being reached because of the localized site of the injection and the possible diffuseness of the neuronal network.

The multisynaptic aspect of these pathways points out another risk involved in the use of ICV injections. There is no *a priori* reason why a multisynaptic pathway would not employ the same transmitter substance at more than one synapse, or that a transmitter substance should not be employed at synapses on opposing pathways. This also must be born in mind when attempting to interpret these results. Thus both methods of application have positive and negative aspects to them and can be used effectively together.

In this first stage of the study only the more diffuse method of application was used, as it was thought best to flood a large area of the CNS with the synaptically-active substances so that if there were responsive synapses they would be affected even if not located in the immediate vicinity of the point of application. It is gratifying that this method has produced results that are readily interpretable in terms of the model of Bligh (1979) (Fig 2.1b). However, a few questions remain (particularly in

regards to the increased $\dot{V}O_2$ following administration of Bi and its relation to thermoregulatory heat production and/or activity of the animal) that now beg the use of more specific methods of administration.

In all previous reports by Bligh (eg. Bligh *et al* 1971) ICV saline (injected as a control, since all of the other drugs are dissolved in it) was found to have no effect on thermoregulation in sheep at high, low or thermoneutral ambient temperatures. I found no effect on effector functions in the hot, although core temperature did rise. This has been attributed to the fact that the animal did not have a full coat at the time of the control experiments, and although effector activities had stabilized at the time of injection core temperature had not and was still rising. At 3° C ICV saline had no effect on respiratory frequency or core temperature but did cause a small but statistically significant rise in $\dot{V}O_2$ lasting about 30 min. This is in conflict with all of the previous reports of no effect on thermoregulation in sheep by Bligh, but supports the findings of Feldberg *et al* (1970) that ICV saline caused shivering and hyperthermia in the cat. The difference between this work and the reports by Bligh are interesting but probably not important or physiologically significant as the increase seen in these experiments was short in duration and did not affect the core temperature. The negative results from ICV saline injections at 40° C also tend to support the conclusion that increased $\dot{V}O_2$ in the cold may have been an artifact. Finally, even if there is some significance to this finding relating to an ionic mechanism in thermoregulation the degree and direction of change was small and/or opposite to those found to be significant in the

rest of this study, and thus can be judged inconsequential as a factor in this study.

GABA injected into the cerebral ventricles of animals other than sheep has been shown to affect thermoregulation (Clark 1979) and food intake (Oligiati *et al* 1980). In sheep Bligh *et al* (1979b) and Seoane *et al* (1984) found that large doses of GABA had no effect on thermoregulation or feeding, respectively when injected into the cerebral ventricles, but that relatively small doses of the GABA agonist muscimol induced marked changes in both functions. Both groups of authors proposed that muscimol was probably acting at GABA receptors and that GABA was not producing a response because it is not easily transported from the ventricle into cerebral tissue, it is catabolized more rapidly than muscimol, and its affinity for its receptors is much less than that of muscimol.

The effects of ICV muscimol in this work were in agreement with the results of Bligh *et al* (1979b) but ICV GABA produced results in direct contrast to these other reports. GABA had some inhibitory effect on respiratory frequency in the heat in doses down to 3 µmol, and effects similar in degree, but shorter in duration, to that of muscimol on both heat production and heat loss at high and low T_a at 39 µmol. This is a dose 3 orders of magnitude greater than the dose of muscimol required to produce similar responses, but well below the dose (50 µmol) found ineffective by Bligh *et al* (1979b). The highest ineffective dose of GABA used by Seoane *et al* (1984) was 3.2 µmol, but even this relatively low dose effectively altered the thermoregulatory response in the current study, and 39 µmol of GABA elicited a feeding-like behavior. This feeding activity was more

clearly produced by muscimol and might be related to the increased food intake described by Seaone *et al*(1984) after ICV muscimol in sheep. The reduction in activity, and apparent onset of sleep in some animals, caused by muscimol might possibly be involved in producing the reductions in $\dot{V}O_2$ and respiratory frequency that have been associated with changes in thermoregulation. However, the decrease in thermoregulatory effector activities always preceeded the changes in activity by 20-30 min and thus these two responses are probably not directly related. Apparently ICV GABA has the same effect on thermoregulation in the sheep as muscimol, and as taurine (Bligh *et al* 1979b) and norepinephrine (Bligh *et al* 1971) as an inhibitor of heat production at low T_a and of heat loss at high T_a. This pattern of thermoregulatory responses could relate either to converging inhibitory signals from other centers in the CNS or to reciprocal inhibition between the pathways from warm sensors to heat loss effectors and cold sensors to heat production effectors, or both (Fig 2.1b).

The different responses to GABA between this work and others in sheep remains unexplained. The possibility of a difference between breeds exists since the previous thermoregulatory studies were done on Welsh Mountain sheep, but this seems unlikely since Seaone *et al* found no effect from GABA in sheep of a similar breed to some of those used in this study. One other possible explanation could involve the cannula placement in the ventricle since the final experiments in this study were done with the cannula in a different position than in the earlier work of Bligh (pers comm- see appendix for details). However this seems unlikely since in the early stages of this work the cannulae were placed in the same position used by

Bligh and the administration of GABA was still effective in inhibiting thermoregulatory responses.

If the inhibition of thermoregulatory responses caused by GABA and muscimol were indicative of a natural role of GABA, the ICV injection of the GABA antagonists Bi and picrotoxin might be expected to result in increased heat loss through panting at high ambient temperature and increased heat production at low ambient temperature. In fact, the results of these injections were an overall excitation of both thermoregulatory pathways at both ambient temperatures. It may be surprising that removal of inhibition onto a pathway that presumably has no drive on it would cause an increase in the effector activity. However, if there were a tonic, or low-level, drive on heat production in the warm and heat loss in the cold then this result would not be unexpected, and in fact the existence of the hypothesized reciprocal inhibition between the two pathways might be assumed to be necessary to turn off this inappropriate effector activity.

The initial response of $\dot{V}O_2$ to Bi is for it to double and for shivering to increase at both high and low T_a. After 30 min shivering and $\dot{V}O_2$ decrease in both cases. $\dot{V}O_2$ returns to the control level at low T_a, but stays elevated by about 35% above the control level at high T_a. The cause of this difference remains unexplained in terms of thermoregulatory effectors, however the significance of the observation of an increase in the activity of the animal after administration of Bi must be considered. An increase in overall activity could be responsible for the observed increase in $\dot{V}O_2$. This may be a case in which the use of ICV injections is stimulating multiple actions of physiological systems that are reflected in thermoregulatory

effector activity, thus making the interpretation of the results unclear. It may be that the effect of this substance on $\dot{V}O_2$ is actually a result of a combination of influences.

The bimodal response of respiratory frequency to Bi at high ambient temperature was unexpected, but possibly supportive of the notion of reciprocal inhibition. This response can be explained if the original model of Bligh (Fig 2.1b) is modified such that the inhibitory synapse from the heat loss pathway onto the heat production pathway acts at a point before the branching of the reciprocal inhibitory signal (Fig 2.17). This explanation also requires the diffusion of Bi from the ventricles to these two populations of GABA receptors to be different so that it reaches the inhibitory neurons acting on the heat production pathway first. Under these conditions $\dot{V}O_2$ would increase as Bi blocks the GABA mediated inhibition of heat production. As $\dot{V}O_2$ increases the inhibition of heat loss would increase causing respiratory frequency to drop. When the Bi reaches the GABA receptors on the heat loss pathway the inhibition would be blocked and respiratory frequency would rise. Of course this scheme is highly speculative and there are probably any number of other reasonable explanations including nonthermoregulatory related phenomenon. Pretreatment of the sheep with Bi before injecting GABA or muscimol blocked the normal inhibitory effects of these substances on respiratory frequency at high T_a and $\dot{V}O_2$ at low T_a. This at least verifies that Bi is acting at GABA receptors and removing an inhibitory drive on the thermoregulatory mechanisms. This result suggested that if, as hypothesized, the inhibitory effects of 5-HT and CCh on $\dot{V}O_2$ at low T_a and

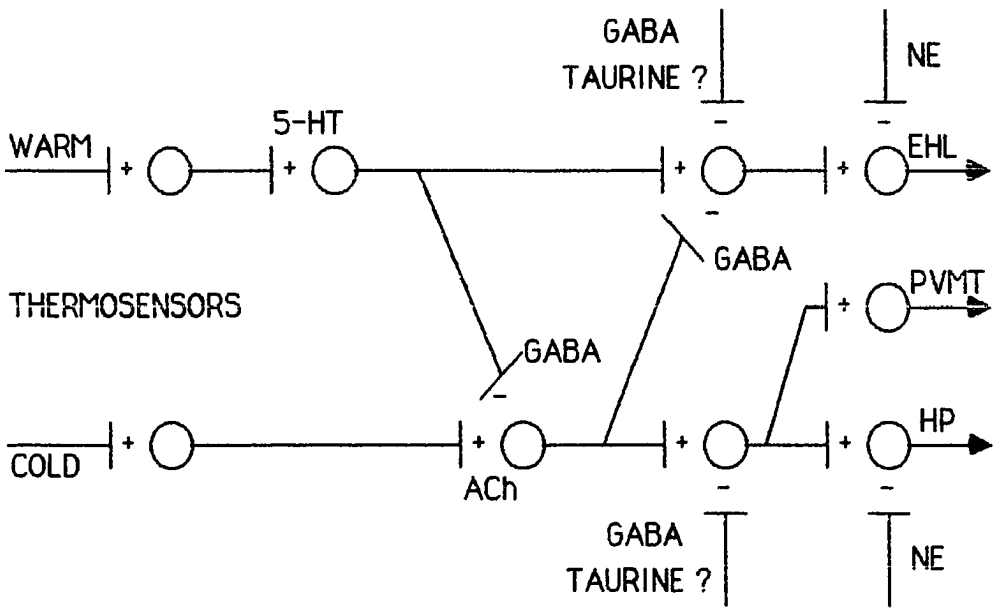

Figure 2.17. A neuronal model depicting the role of GABA as the terminal transmitter of reciprocal inhibition between the warm sensor to heat loss and cold sensor to heat production pathways in the brain of sheep. GABA may also act as a transmitter of converging inhibition from other brain centers. Symbols and abbreviations as in Figure 2.1.

respiratory frequency at high T_a, respectively were due to reciprocal inhibition mediated by GABA, then pretreatment with Bi should remove this inhibition and allow the activation of both heat production and heat loss simultaneously.

This is exactly what happened when this experiment was performed at 41° C with CCh. The increased $\dot{V}O_2$ could be attributed to the effects of Bi discussed earlier, and thus may not be directly associated with thermoregulation. However, in this case the $\dot{V}O_2$ remained 60% elavated for 60 min rather than falling to 35% above the control level after only 30 min. This suggests that the high $\dot{V}O_2$ after the administration of CCh in this experiment was due, at least in part, to the action of CCh, an effect not seen after an injection of CCh alone.

Pretreatment with Bi at low T_a before administration of 5-HT produced similar, but less clear, results to those just discussed. The inhibition of $\dot{V}O_2$ by 5-HT, which normally occurs immediately, was delayed by 30 min. During the 30 min period after injecting 5-HT the trace of $\dot{V}O_2$ followed the normal course seen after injecting Bi alone, returning to the control level and remaining stable after 30 min. This suggests that the delayed decrease of $\dot{V}O_2$ is not related to any effect of increased activity on $\dot{V}O_2$, and thus is probably indicative of changes in a thermoregulatory response.

The 67% increase in respiratory frequency for 25 min after the injection of 5-HT followed the pattern produced by Bi alone, and was probably not a response to 5-HT. However, the sudden onset of panting for a 15 min period 30 min after the 5-HT injection is not normally associated

with ICV injections of either Bi or 5-HT by themselves. This appears to be caused by Bi blocking a GABA mediated inhibition which allows 5-HT to excite the heat loss pathway. The short duration of this response is probably due to the normal inactivation of Bi but the long delay in the onset of the excitation remains unexplained. The onset of panting coincided with the beginning of the 5-HT mediated inhibition of $\dot{V}O_2$ indicating that the effects of Bi on the heat production pathway had worn off. The possibility of a differential rate of diffussion of Bi, discussed earlier, as a likely factor in the delay does exist. However, the much longer delay in this case (25 min versus 5 min) and the short period of the response after its onset do not support this explanation.

Thus it appears that GABA is probably the terminal inhibitory neurotransmitter of reciprocal inhibition between the two major thermoregulatory pathways, but this does not rule out its likely role as a neurotransmitter of converging inhibition from other areas of the CNS (Fig 2.17).

CHAPTER 3

SIMULATIONS OF PHARMACOLOGICAL EXPERIMENTS ON THE PHYSICAL MODEL

Introduction

In the previous two chapters I have shown; 1) that a temperature insensitive reference signal is not a requirement for thermoregulation, 2) that thermoregulation may depend upon the interactions of opposing responses of sensor signals with reciprocal inhibition between the efferent signals to opposing response effectors and 3) that gamma-aminobutyric acid (GABA) may be the terminal inhibitory neurotransmitter of reciprocal inhibition between the two thermoregulatory pathways. Numbers 1 and 2 were demonstrated using a physical model of thermoregulation (see Chapter 1), while number 3 was shown with pharmacological synaptic interference studies on the conscious sheep (see Chapter 2).

While the physical model discussed in Chapter 1 did not accurately simulate all of the physiological mechanisms of thermoregulation in the sheep, it did describe the hypothesized connections of the neuronal network between the thermal sensors and thermal effectors in the pre-optic anterior hypothalamus of the sheep as originally deduced by Bligh and his colleagues from results of experiments similar to those described in Chapter 2 (see Bligh 1979). Even though the neuronal aspect of the model was not precise in terms of the thermoneutral and set-point temperatures of the system or in terms of the gains of the effectors in relation to the sensors, the pharmacological experiments of Chapter 2 should be able to be simulated with the physical model. These simulations may not be quantitatively accurate, but they should reproduce the general trends of the relationships

between the sensors, the effectors and core temperature seen in the pharmacological manipulations of Chapter 2.

In this final chapter I will discuss attempts to simulate some of the experiments discussed in Chapter 2 on the physical model described in Chapter 1 and interpret these results in terms of their support for the model.

Methods

The physical model described in Chapter 1 was used in these simulations. Figure 3.1 is a modification of the block diagram (Fig 1.9) indicating the hypothesized points of action of some of the neurotransmitters of the neuronal model, as discussed in Chapter 2 (Fig 2.1b). External inputs were made at these points to simulate intracerebroventricular (ICV) injections of 5-hydroxytryptamine (5-HT), Carbamylcholine (CCh) and gamma-aminobutyric acid (GABA).

5-HT

An external input of +2.0 V was made into the Σ stage of the heat sensor to heat loss pathway (Fig 3.1) for 5 min to simulate the ICV injection, or release, of 5-HT in the sheep. Recordings of effector activity and T_{in} were made at 1 min intervals during this period, and for the 5 min intervals before and after. These experiments were performed at T_{out} = 32° C and 30° C to simulate thermoneutrality and cold stress respectively.

CCh

An external input of +2.0 V was made into the Σ stage of the cold sensor to heat production pathway (Fig 3.1) for 5 min to simulate the release of acetylcholine (ACh), or injection of the ACh agonist CCh in the sheep. Results were recorded as described above. These experiments were performed at T_{out} = 32° C and 35° C to simulate thermoneutrality and heat stress respectively.

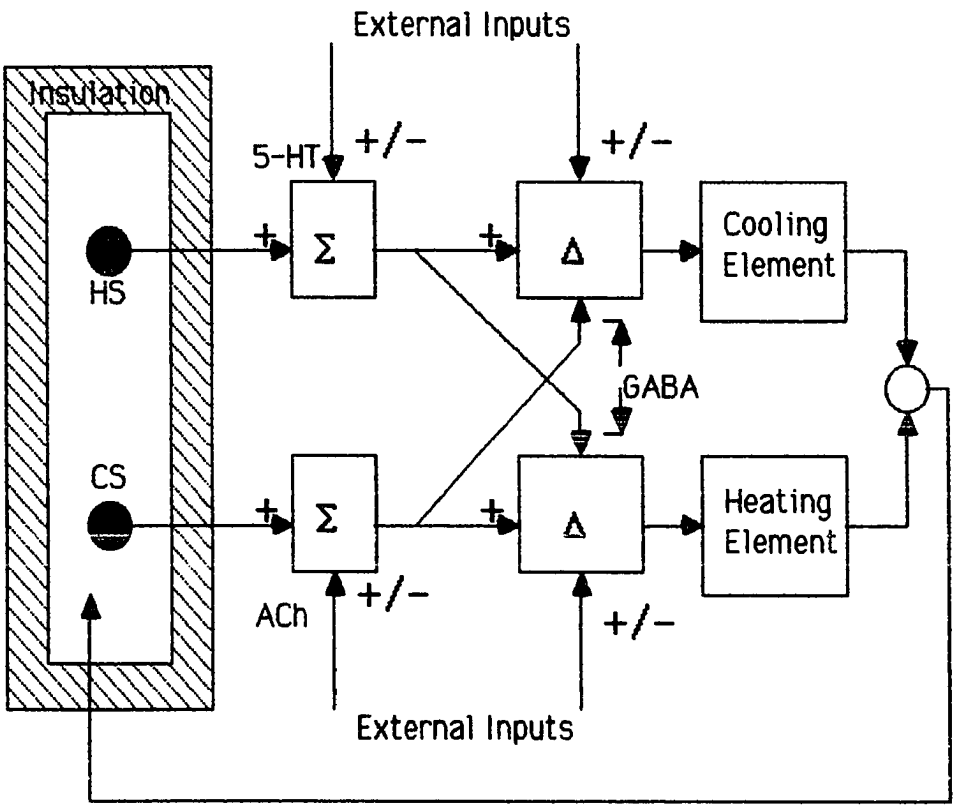

Fig 3.1. Block diagram of the physical model indicating the hypothesized points of action of some of the neurotransmitters of the neuronal model. HS = heat sensor, CS = cold sensor, Σ = summer, Δ = differencer, Heating Element = 100, 10W resistor, Cooling Element = thermoelectric peltier device, (+) = excitatory input, (-) = inhibitory input.

GABA

External inputs of -2.5 V were made simultaneously into both Δ stages of the model (Fig 3.1) for 5 min to simulate the ICV injection of GABA in the sheep. Results were recorded as described above. These experiments were performed at T_{out} = 30° C, 32° C and 35° C to simulate cold stress, thermoneutrality and heat stress respectively.

Bicuculline

Bicuculline-methiodide (Bi) is a specific antagonist of GABA, and was used in the pharmacological experiments to block the effects of endogenous release of GABA in the sheep. In order to simulate the ICV injection of Bi into the sheep, the reciprocal inhibitory connections between the two sensor to effector pathways (Fig 3.1) had to be removed from the model. This was done by disconnecting the inverting input to each Δ stage 741 operational amplifier (Fig 1.7) and connecting them to ground through the 10K resistor. Results were recorded as described above.

Results

5-HT

Figures 3.2a and 3.3a show that at both T_{out} = 30° C and 32° C T_{in} dropped about 0.4° C during the period of the simulated injection. T_{in} returned to the preinjection levels immediately after removal of the external input. At T_{out} = 30° C the heat production effector was active before and after the injection and became inactive during the injection, while the heat loss effector became active during the injection (Fig 3.2b). At T_{out} = 32° C both effectors were inactive before and after the injection, with the heat loss effector becoming active during the injection (Fig 3.3b).

CCh

Figures 3.4a and 3.5a show that at T_{out} = 32° C and 35° C T_{in} rose about 0.5° C and 0.6° C respectively during the period of the simulated injection and returned to approximately the preinjection levels immediately after removal of the external input. At T_{out} = 32° C both effectors were inactive before and after the injection, with the heat production effector becoming active during the injection (Fig 3.4b). At T_{out} = 35° C the heat loss effector was active before and after the injection and became inactive during the injection, while the heat production effector became active during the first minute of the injection and then became inactive for the rest of the period (Fig 3.5b).

GABA

At the thermoneutral temperature, T_{out} = 32° C, both effectors were inactive before and after the injection. T_{in} and the effector activities were

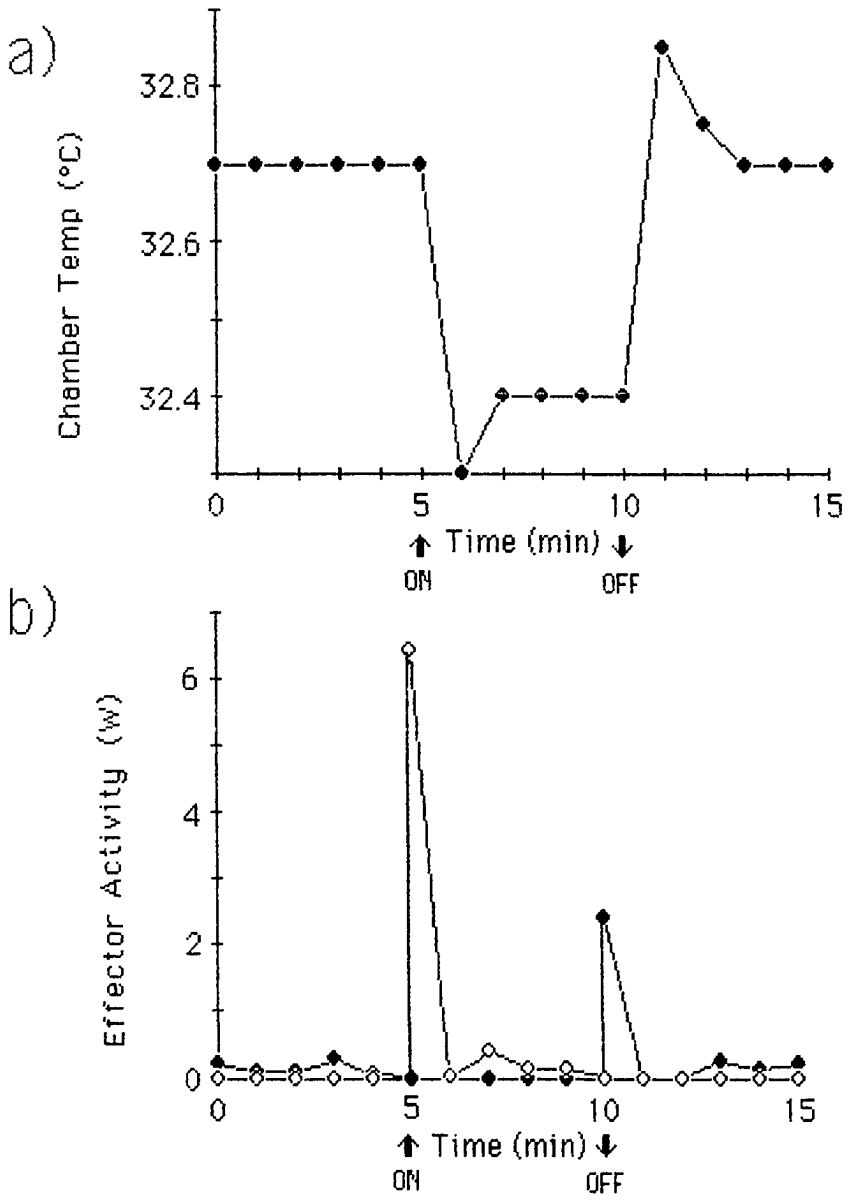

Fig 3.2. Simulation of the effects of an ICV injection of 5-HT on a) chamber temperature (T_{in}) and b) heat production (●) and heat loss effector (o) activities. T_{out} = 30° C. On = beginning of external input. Off = removal of external input.

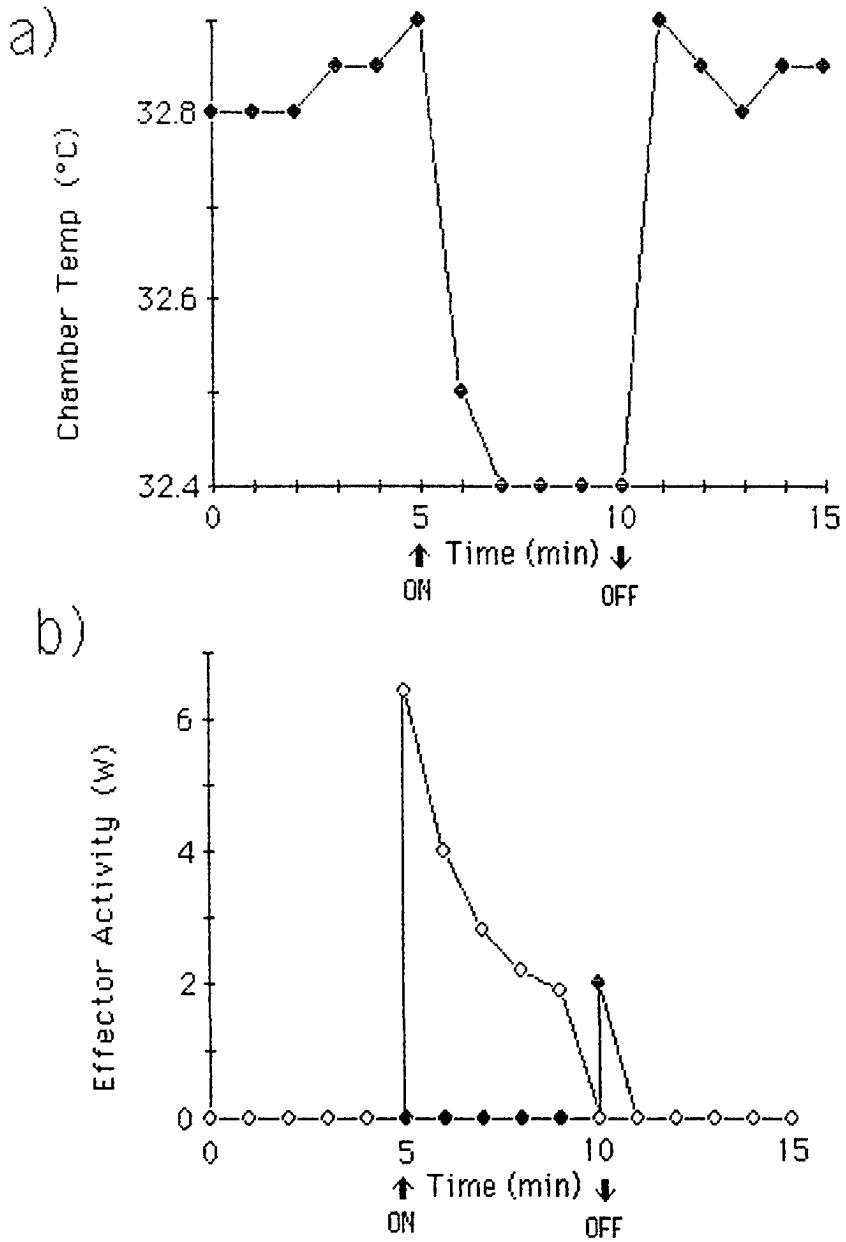

Fig 3.3. Simulation of the effects of an ICV injection of 5-HT on a) chamber temperature (T_{in}) and b) heat production (●) and heat loss effector (o) activities. T_{out} = 32° C. Symbols as in Figure 3.2.

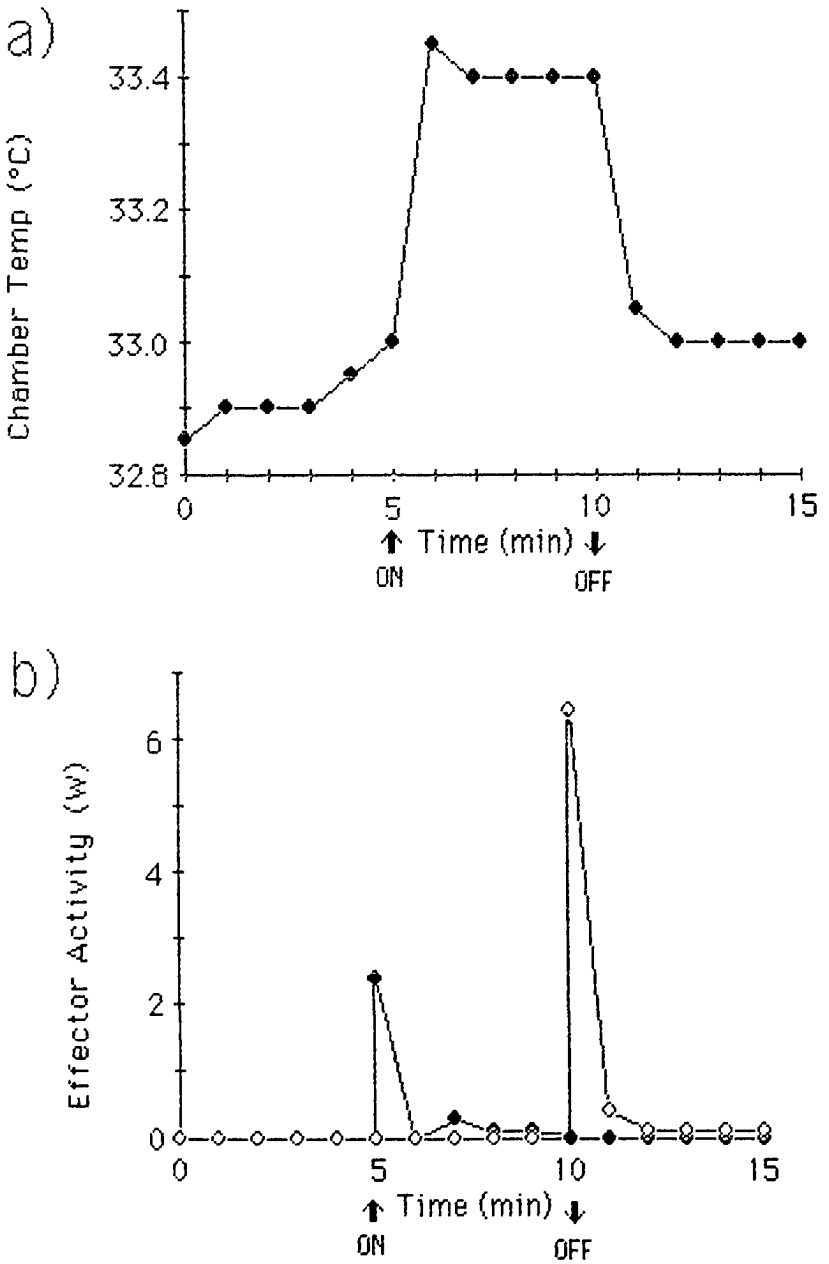

Fig 3.4. Simulation of the effects of an ICV injection of CCh on a) chamber temperature (T_{in}) and b) heat production (●) and heat loss effector (o) activities. T_{out} = 32° C. Symbols as in Figure 3.2.

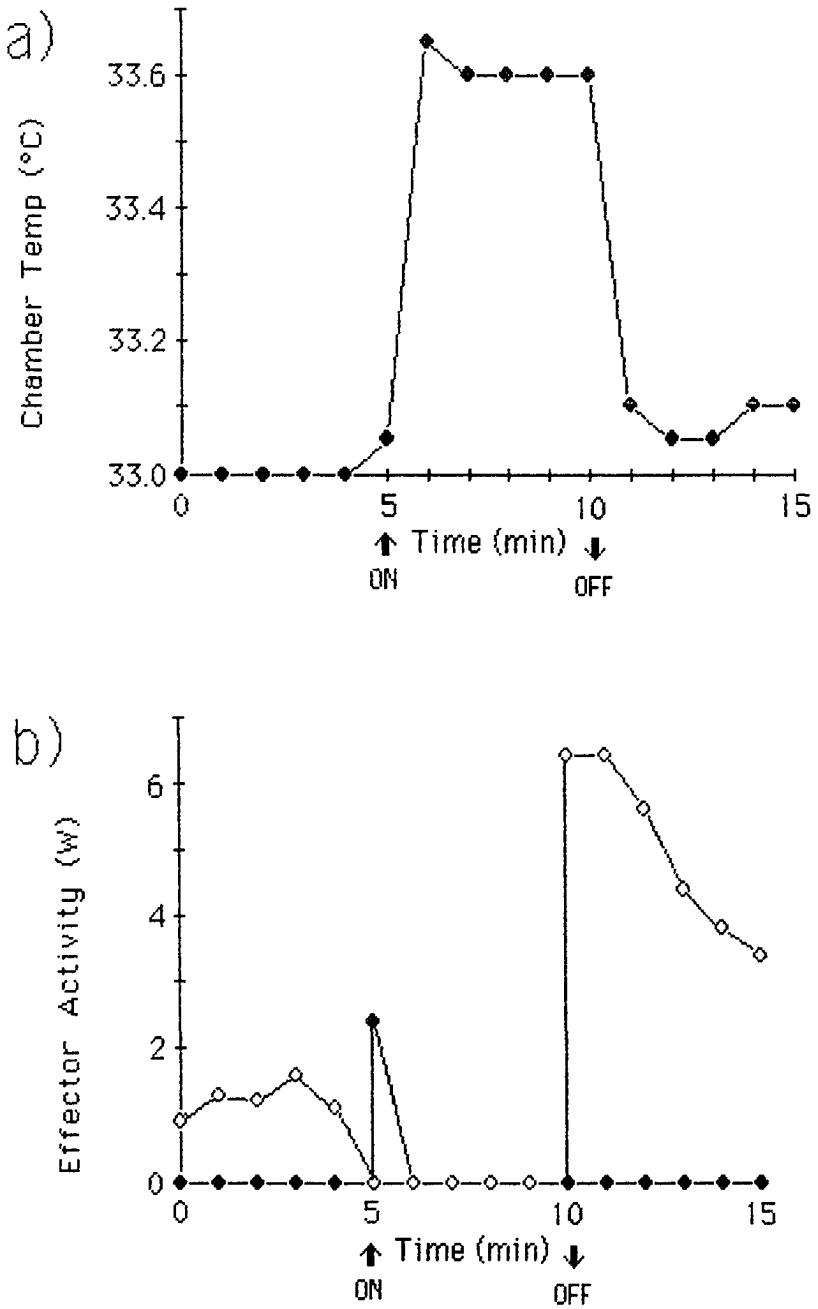

Fig 3.5. Simulation of the effects of an ICV injection of CCh on a) chamber temperature (T_{in}) and b) heat production (●) and heat loss effector (o) activities. T_{out} = 35° C. Symbols as in Figure 3.2.

unaffected by the external input.

At T_{out} = 30° C the heat production effector was active before and after the inhibitory input, and became inactive during the inhibition (Fig 3.6b). T_{in} fell 0.1° C during the inhibiton, and returned to the preinjection level after removal of the external input (Fig 3.6a).

At T_{out} = 35° C the heat loss effector was active before, during and after the inhibitory input, but its activity was depressed during the period of application of the external input (Fig 3.7b). T_{in} rose 0.1° C during the inhibition, and returned to the preinjection level after removal of the external input (Fig 3.7a).

Bicuculline

Both effectors became fully active at T_{out} = 30° C, 32° C and 35° C upon implementation of the rewiring of the circuitry described under Bicuculline in the Methods section of this chapter.

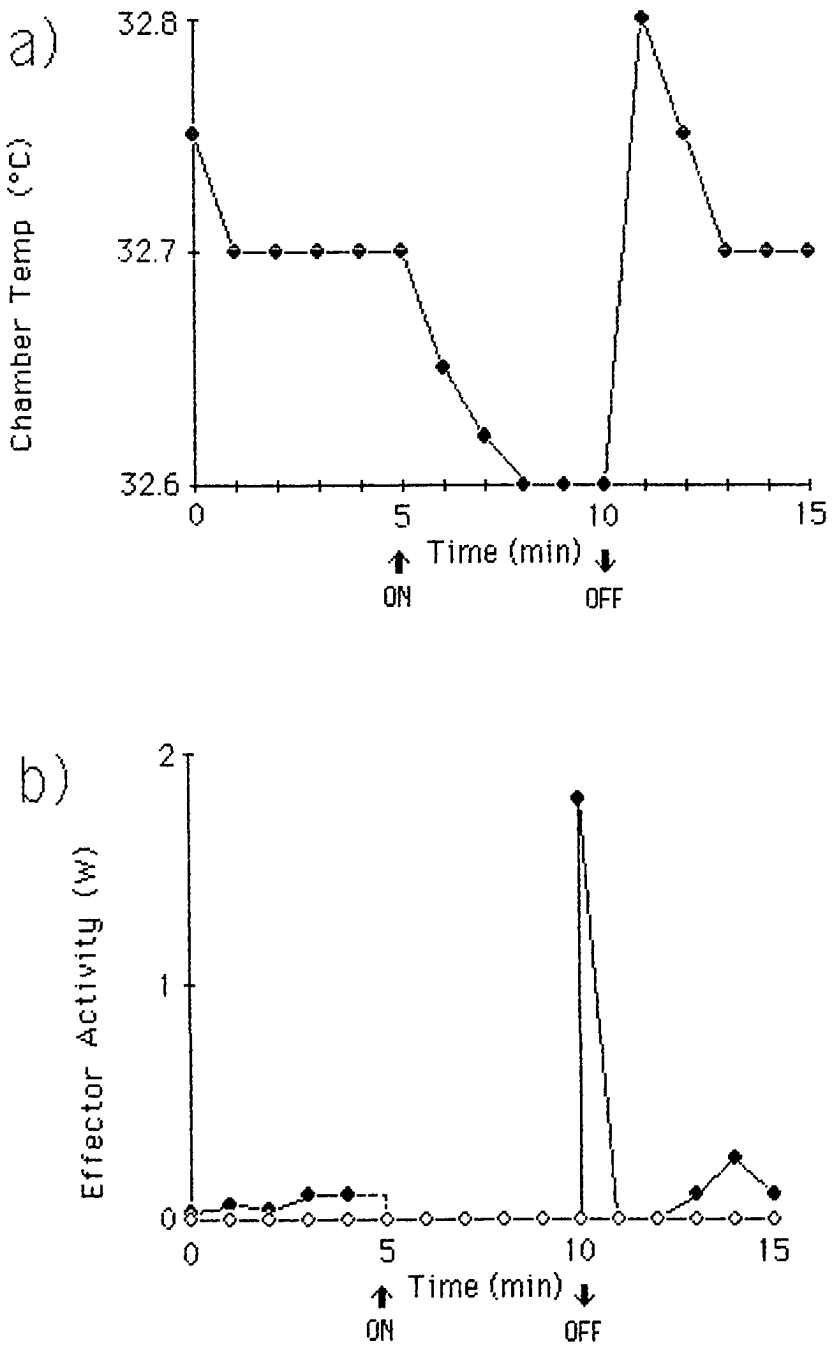

Fig 3.6. Simulation of the effects of an ICV injection of GABA on a) chamber temperature (T_{in}) and b) heat production (•) and heat loss effector (o) activities. T_{out} = 30° C. Symbols as in Figure 3.2.

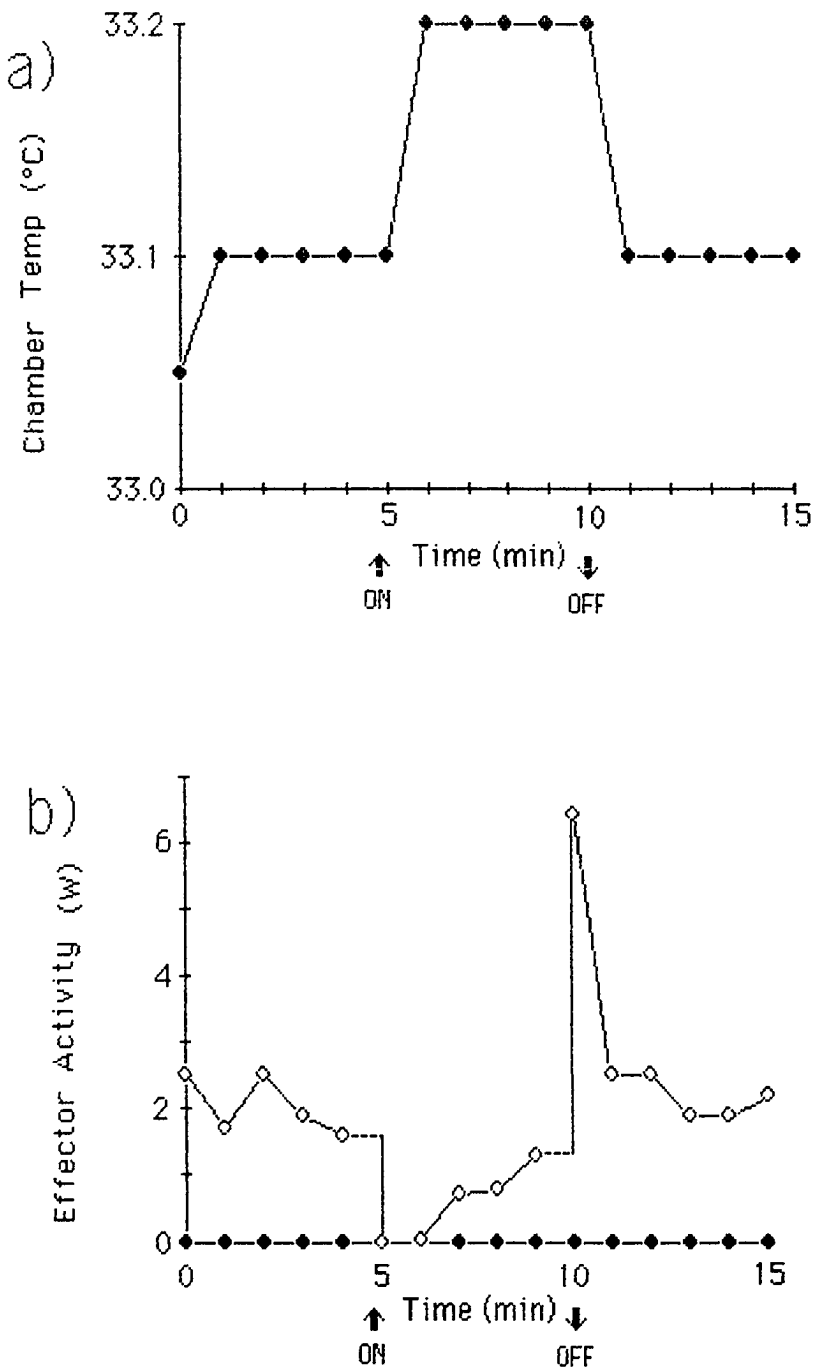

Fig 3.7. Simulation of the effects of an ICV injection of GABA on a) chamber temperature (T_{in}) and b) heat production (•) and heat loss effector (o) activities. T_{out} = 35° C. Symbols as in Figure 3.2.

Discussion

The results of adding excitatory or inhibitory inputs to points in the physical model corresponding to sites in the neuronal model at which 5-HT, ACh and GABA are hypothesized to be neurotransmitters produce results qualitatively similar to those found after the ICV injection of these substances into the sheep. The results cannot be compared quantitatively because the physical model is not a quantitative representation of the mammalian thermoregulatory system. The effects of different doses and the kinetics of the uptake and metabolism of drugs injected into the brain were not included in the physical model. The inputs used to simulate the injections of drugs were turned on and off instantaneously and remained at a constant level for the entire duration of their action. This situation was not accurate and led to the overshoots of effector activity and temperature changes seen at the initiation and termination of the stimulus in some cases.

The results of the simulations of the bicuculline experiments are not even qualitatively representative of the neuronal model, because of the high gains used in this version of the physical model. The high gains necessary to maintain temperature near the setpoint without a large amount of drift above and below it caused the effectors to be fully active when T_{in} was 0.2° C above or below the setpoint (Fig 1.11). When the reciprocal inhibition was removed, as in the case of the bicuculline simulations, the heat loss effector was fully active at $T_{in} > 29.2$° C, and the heat production effector fully active at $T_{in} < 36.2$° C. Thus, when the reciprocal inhibition was

removed at T_{in} near the setpoint both effectors became fully active and remained so until the chamber temperature changed almost ±3.5° C. The inclusion of peripheral temperature sensors in the future should allow the model to maintain a regulated temperature with little drift without the high gains of the present version. Such a model should more accurately simulate the mammalian system and allow for the simulation of the bicuculline experiments. In a preliminary test, without the peripheral sensors, a model with a gain of 0.5 did qualitatively simulate the bicuculline experiments such that both effectors were partially active simulataneously. If $T_{in} <$ 32.7° C the heat production effector was more active than the heat loss effector, while if $T_{in} >$32.9° C the heat loss effector was more active.

The present version of the physical model is capable of qualitatively simulating the results of synaptic interference studies performed on animal models. Future refinements of the model should allow for more accurate simulations, and in the meantime these results do support the neuronal model of thermoregulation proposed by Bligh (1979) and discussed in Chapters 1 and 2 of this dissertation.

CONCLUSIONS

1. A temperature insensitive reference signal is not a requirement for thermoregulation in a physical or neuronal model.

2. Thermoregulation may depend upon the interactions of opposing responses of sensor signals with reciprocal inhibition between the efferent signals to opposing response effectors.

3. Gamma-aminobutyric acid may be the terminal inhibitory transmitter of reciprocal inhibition between the two thermoregulatory pathways.

4. The results of pharmacological experiments on animal models and simulations of these experiment on a physical model are qualitatively similar. These results support the neuronal model of Bligh (1979).

APPENDIX

AN IMPROVED METHOD FOR CANNULATION OF THE LATERAL CEREBRAL VENTRICLE OF THE SHEEP

Introduction

At least three descriptions of the surgical technique for the chronic cannulation of the lateral cerebral ventricles of sheep exist in the literature (Palmer1959, Barton *et al*1969, and Seoane1981). The coordinates for the placement of the cannula are different in all three of these reports. They range from "8 mm posterior to the frontal-parietal bones suture and 8 mm lateral to each side of the midline" (Seoane1981) to "5 mm lateral to the mid line and 5 mm caudal to the fronto-parietal suture" (Palmer1959) to the placement by Barton *et al*(1969) (pers. comm.) 4 mm lateral to bregma (the intersection of the sagittal and coronal sutures). The cannulations described by Barton *et al*(1969) and Palmer (1959) were done on Welsh Mountain wethers while Seoane describes the cannulation of crossbred (Leicester x Dorset x Suffolk) (Seoane1981) and Suffolk (Seoane *et al* 1984) wethers.

We used the technique and the coordinates of Barton *et al*(1969) (see above). The sheep in this study were crossbred wethers (including Columbia x Targhee, FinDorset x Suffolk and Ramboulett x Suffolk). Placement of the cannula 4 mm lateral to bregma was unsatisfactory. In animals over 3 years old it was difficult, and sometimes impossible, to cannulate the ventricle, and in younger sheep (1-3 years old) it was difficult to keep the cannula patent for longer than 2 weeks.

A radiograph of a cannulated Welsh Mountain sheep skull (Fig A.1) shows clearly the cannula placement in the thin caudal tail portion of the

Fig A.1 A radiograph of a cannulated Welsh Mountain sheep skull.

lateral ventricle. Post-mortem inspection of skull cross-sections from some of our cannulated sheep showed the cannulae in approximately the same thin tail section, about 10 mm caudal to the main body of the ventricle. We felt that this may have been responsible for our lack of success in placing and maintaining the cannulae and thus decided to move the cannula rostrally 10 mm. This report describes the procedure.

Methods

Before surgery cannulae were constructed from 4"18 gauge stainless steel hypodermic needles (Fig A.2a). After the tip was removed the end of the cannula was closed with silver solder and filed smooth. A side opening was made 3-4 mm from the tip with a small round file. Measurements were made of the cannula and guide sleeve to determine at what point the cannula would protrude 28 mm below the dura mater (the approximate area of the ventricle). A hollow stainless steel bolt and four rubber washers (3 mm diameter) were placed on the cannula.

Sheep were deprived of food and water for 24 h prior to surgery. A polyethylene catheter was placed in a jugular vein under local anesthesia (lidocaine-hydrochloride, 2%). General anesthesia was induced with sodium pentobarbital (20-25 mg/kg) administered via the catheter. Anesthesia was maintained with halothane. The animal was placed on a surgical table in ventral recumbency and intubated. The head was secured in a specially designed vise so that the parietal region was horizontal. The head region was then shorn and scrubbed with a topical antiseptic (povidone-iodine 10%) , and the animal was draped with sterile surgical drapes.

A 40 mm midline incision was made caudal to the ears and the tissue cleared and retracted to expose an area of skull about 30 mm in diameter. 2-3 ml epinephrine (1:100,000) was dripped into the incision to control bleeding. A point was marked 4 mm lateral to the midline and 10 mm rostral to bregma. A hole was drilled through the bone, but not through the dura mater, with a 4 mm bone drill bit driven by a 1/4" electric hand drill

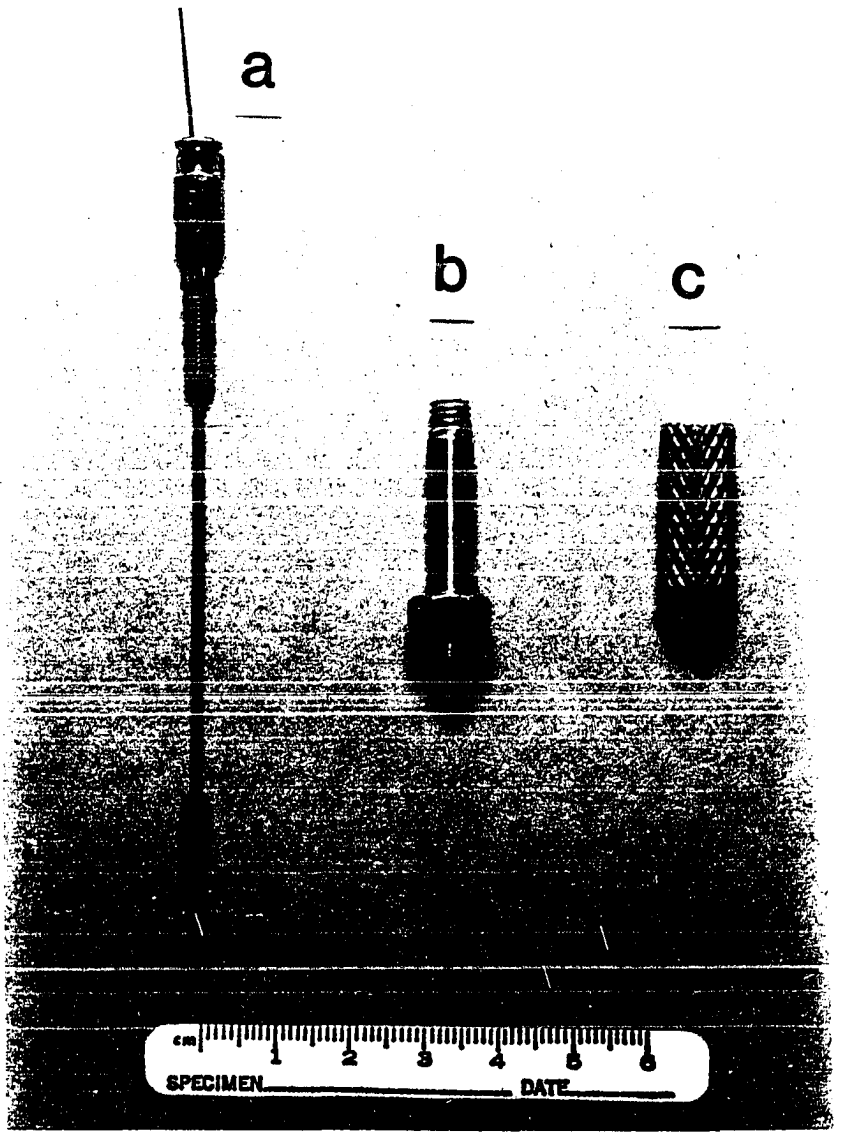

Fig A.2. Photograph showing the a) cannula, b) guide sleeve and c) stainless cap used to cannulate the sheep.

with a 1.3 m flexible extension. The hole was tapped with a 6 mm x 1 bone tap and the threaded guide sleeve (Fig A.2b) was screwed into the hole. Four or five holes (6 mm deep) were drilled into the skull around the base of the cannula holder (about 10 mm away) with a no. 39 drill bit. A no. 6 x 3/8" panhead stainless steel self tapping screw was placed in each hole. A mound of dental acrylic was built around the guide and screws, thus anchoring the guide to the skull.

A 1 cc glass syringe (without shaft) was placed in the luer lock of the cannula and filled with sterile saline. The cannula tip, washers and bolt were inserted into the guide and the cannula was inserted rapidly to the premeasured depth. The correct placement of the cannula into the lateral ventricle was determined by observing the change in height of the saline column as it entered the cerebral ventricle. An initial pressure increase was usually noted about 4 mm above the ventricle when the cannula entered the sulcus lying above the corpus calosum. The ventricle was identified by further pressure increase accompanied by an observable pulse. The cannula was locked in position by screwing the bolt into the threaded interior of the guide sleeve thus compressing the rubber washers around the cannula shaft. The cannula was cut off 4 mm above the guide sleeve and sealed with a blind-ended polyethylene sleeve. A stainless steel cap was then screwed over the guide sleeve for further protection (Fig A.2c). Nitrofurazone powder was applied to the wound. The periosteum was brought together over the dental acrylic mound to prevent new periostial bone fromation beneath the structure. The muscle and connective tissue layers were closed around the guide with single interrupted 000 chromic gut sutures and the

skin sutured with single mattress 0 silk . The procedure required 2-3 hrs. After surgery the animal received liquamycin LA-200 (oxytetracycline-Pfizer) (3mg/pound diluted in an equal volume of sterile saline) intravenously. A helmet constructed of a closed cell rigid foam was taped to the head over the cannula, and the animal was returned to the pen. The cannula was kept patent by flushing with 0.4 ml saline every 2 days. Proper placement of the cannula was verified after 1 week by subjecting the animal to 41° C and injecting norepinephrine (10 µmol) into the cannula. Proper placement was indicated by an immediate inhibition of panting (Bligh *et al* 1971).

Discussion

This technique has proved satisfactory for our needs, and movement of the cannula to the new position (10 mm rostral to bregma, and 4 mm lateral to the midline) has allowed us to maintain cannulae for 4 months. The reasons behind the discrepancies in cannulae placement discussed in the Introduction remain obscure. Anatomical differences between breeds of sheep may partially explain the discrepancy, but three points make this explanation questionable. First, there was a large discrepancy between the cannula locations in our wethers and those of Seoane (1981 and 1984) which were similar crossbreeds. Second the radiograph showing placement of the cannula in a Welsh Mountain wether and our postmortem investigation of cannulated crossbred sheep skulls indicate that the ventricles are in essentially the same location relative to the skull regardless of breed. Finally, cannula placement 5-8 mm caudal to bregma (Palmer1959 and Seoane1981) would be caudal to the ventricles.

This is a useful technique for determining the physiological effects of centrally applied neuroactive substances. When the correct coordinates for cannula placement are known it is also a simple technique.

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