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Monoamines, Pyrogens and Cations: Their Actions on Central Control of Body Temperature

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I. Introduction

THIRTEEN productive years have elapsed since von Euler reviewed the pharmacology of thermoregulation in this journal (198). It is not my intention to attempt a similar general survey of all the recent literature. To do so would be a daunting task and, in any case, the end result would very likely be far too lengthy. Instead of such a general survey I have chosen to review critically three aspects of the actions of drugs on the parts of the brain involved in regulating body temperature. First, attention will be concentrated on the possible role of the brain monoamines as neurotransmitters in this regulating system. Next, the disturbance of temperature control by pyrogens and prostaglandins will be considered. Finally, the possible involvement of sodium and calcium ions in temperature regulation will be discussed.

There have been two recent reviews of the pharmacology of thermoregulation elsewhere (131, 178) and pharmacological aspects have been included in publications covering thermoregulation generally (23, 28, 36, 105, 113, 168).

II. Temperature Regulation as a Control System

In considering such a complex system as that for regulating body temperature it is helpful to have a conceptual framework or model on which to "hang" various ideas. One useful model is based on control-systems engineering and consists of a controller with negative feedback loops to provide information on the thermal state of the body. Such models can be quite complex (*e.g.*, 101) but for the present purpose the much simpler block diagram shown in figure 1 will suffice.

The "controlled system" is the body itself, while the "disturbance" is any environmental factor which alters heat exchange and/or the heating effect of exercise. There is not yet agreement as to which body temperature or combination of temperatures constitutes the "controlled variable," but whatever temperature is concerned, it will be subject to disturbances and these disturbances in turn will activate the "feedback elements" or temperature sensors.* Such sensors are known to be present in the skin as actual end organs and similar end organs presumably exist at other somatic sites known to be thermosensitive (112). There are also neurons in the central nervous system which behave like temperature sensors (110, 113). The latter form part of the "black box" in figure 1A. Also contained in the black box will be other neurons (fig. 1B) concerned with integrating the thermal information from the sensors, establishing a "set-point" and finally issuing efferent signals to counteract the disturbance. It is still not clear whether the comparator which establishes the set-point incorporates an internal reference (or thermostat) or operates by comparing two types of feedback, one from warm-sensitive elements and the other from cold-sensitive elements (113). The efferent signals govern the "control actions" which comprise autonomic mechanisms such as skin vasocon-

*In this review the term "sensor" is used to denote what would be called a receptor in physiological parlance; "receptor" is used only in its pharmacological sense.

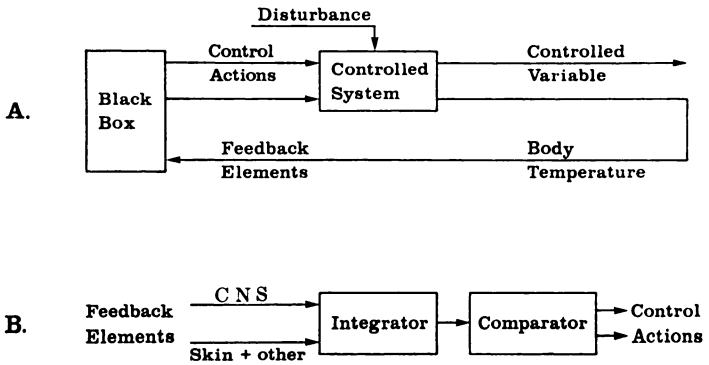

FIG. 1. A. Block diagram of a simple feedback model for the control of body temperature. B. Hypothetical components for the "black box" in A. The abbreviation used is: CNS, central nervous system.

striction, shivering, piloerection, panting or sweating and behavioural responses such as basking, burrowing and huddling.

Anatomically, many of the neurons making up the circuits in the black box are to be found in the preoptic area and the anterior and posterior hypothalamus. Furthermore these neurons are medially situated so they may be accessible to drugs by diffusion through the walls of the third ventricle.

Functionally, the model shown in figure 1 is obviously only an approximation to reality and the actual circuitry is largely unknown. Unit recordings have revealed the presence of several categories of neurons (113) which are presumed to be components of the black box, but a direct proof of this presumption is lacking. Despite this almost total lack of understanding of the neuronal circuitry, a host of investigators (including myself) have attempted to analyse the working of the black box by introducing candidate transmitter substances or other drugs into the cerebral ventricles and observing the control actions or resultant changes in body temperature. Astonishingly, co-ordinated autonomic and behavioural responses are often observed when such experiments are made on a conscious animal. A plausible explanation for these surprising results will be offered after the relevant literature has been reviewed (section III E).

III. Possible Transmitter Substances and Their Central Effects on Temperature

A. Historical

As early as 1943 it had been found that injections of adrenaline into the cerebral ventricles of rabbits raised their body temperature by several degrees (199). By 1961, von Euler (198) was able to make the following prediction: "It is currently believed that catecholamines, 5-HT, or both may be mediators of activity of brain stem structures and involved in the regulation of somatic, autonomic, and cortical activity. It is inviting, therefore, to think that these putative central neurohumors, as well as drugs interfering with their actions of metabolism, might also exert an influence on the setting mechanism of the body thermostat."

The first experiments, which could be said to bear out von Euler's suggestion were published in 1963 by Feldberg and Myers (78). They injected microgram doses of adrenaline, noradrenaline (NA) or 5-hydroxytryptamine (5-HT) into the cerebral ventricles of cats and observed hypothermia with the two catecholamines and hyperthermia with 5-HT. As a consequence they put forward the bold hypothesis that body temperature is controlled by a balance of these opposing actions due to the relative rates of release of catecholam-

ines and 5-HT in the hypothalamus. Much of the recent work which is reviewed below can be said to have been motivated by the Feldberg-Myers hypothesis.

B. Presence of monoamines in the hypothalamus and brain stem

Analyses of the content of NA and 5-HT show wide variations between different regions of the brain in cats and dogs. The highest concentrations of NA are found in the hypothalamus and the area postrema (197). These two areas and the midbrain are also rich in 5-HT (3, 29).

It was the visualization of these monoamines in nerve cell bodies and axon terminals by fluorescence microscopy which pointed to their possible function as neurotransmitters. Dahlström and Fuxe (58) found that nearly all the 5-HT-containing cell bodies were localized in the various raphé nuclei with their axons passing caudally to the spinal cord and rostrally in the median forebrain bundle to many regions including the hypothalamus. No cell bodies containing 5-HT were seen in the hypothalamus itself. The catecholamine-containing cells were more widely scattered through the brain stem but the most dense concentration was in the mesencephalon at the level of the nucleus interpeduncularis. Many of the fibres from these cells also pass up in the median forebrain bundle to the hypothalamus and catecholamine nerve terminals have been seen in three hypothalamic areas: a) preoptic region ventral to the anterior commissure; b) the supraoptic nuclei; and c) the paraventricular nuclei (38, 97). A few scattered catecholamine cell bodies can also be seen near the third ventricle (21, 58).

This fluorescence microscopy has been conducted primarily on the rat, but there have also been studies in cats (134, 166) and human fetuses (165) which show a broadly similar distribution of monoamine neurons. It is unfortunate that a wider variety of species has not been examined in view of the major differences which have been found between species in temperature responses to intraventricular injections of drugs. Nevertheless, the important conclusion is that neurons containing catecholamines and 5-HT seem to be concerned with relaying information to the hypothalamus, rather than with integrative action within the hypothalamus or with efferent fibres leaving it. One obvious source of afferent information is the traffic from the thermal sensors in extrahypothalamic sites such as the spinal cord, medulla and skin. There is some evidence indicating that such a relay of thermal information to the hypothalamus does take place (110, 113), but, with the exception of the work of Weiss and Aghajanian (201), none of this evidence has been concerned with the nature of the transmitter substances involved. This is a point which will be considered again below.

C. Evidence from various species

That the intracerebro-ventricular (ICV) injection of a drug which causes hyperthermia in one species, may cause hypothermia in another and have no action in a third is a situation which is perhaps without precedent in pharmacology. Because of these differences each species will be treated separately. In those species which have been studied extensively, namely cats, rabbits, and rats, the evidence will be presented in tabular form. Although no convincing explanation for the species differences has yet emerged, various possibilities will be discussed in section III D.

1. Cats. The results obtained from experiments in which NA, 5-HT or acetylcholine (ACh) were given to cats by ICV injection or microinjection are summarized in table 1.

With NA there was a consistent fall in body temperature brought about by skin vasodilatation and possibly reduction in heat production. The site of action was in the preoptic/anterior hypothalamic (PO/AH) region (see table 1 for refer-

TABLE 1

Summary of data from cats

Drug	Technique	Ambient Temperature or State of Animal	Effect on Control Actions	Effect on Body Temperature	Remarks	References
NA*	ICV NA	20–22°C	Vasodilatation No panting Metabolism?	↓ by 1–2°C		79
	ICV NA	Shivering due to pyrogen	Shivering stopped	↓		79
	Microinjection of NA in PO/AH		As for ICV	↓	Dose-dependent response	80, 175
	Microinjection into posterior or ventromedial hypothalamus		None	None		175
	ICV phenoxybenzamine	19–22°C		Weak ↑		86
	ICV imipramine	19–21°C		↓ 1.7°C	Response reduced by prior NA depletion	49
	Depletion with reserpine			↓	Only with first dose	13
	ICV 6-hydroxydopamine			↓ 4.4°C	Weaker response to second dose	130a
5-HT	ICV 5-HT or microinjection	20–22°C	Vasoconstriction, shivering or vasodilatation, tachypnoea	↑ or ↓ or nothing	Animal sedated	12, 79, 80, 130, 202
	ICV tranylcypromine		Shivering	↑		77
ACh	ICV nicotine	22°C	Vasodilatation, panting	↓ 1–2°C	Blocked by hexamethonium, or mechamylamine	100
	ICV carbachol	22°C	Vasoconstriction, shivering	↑	Blocked by atropine	100
	ICV eserine	36–38°C	Panting			93
	ICV eserine	6–8°C	Shivering increased	↑ 1°C		93
	Microinjection of carbachol	21°C		↓ or ↑	Effect varied with dose and site	176

* The abbreviations used are: NA, noradrenaline; ICV, intracerebro-ventricular; PO/AH, preoptic and anterior hypothalamic; 5-HT, 5-hydroxytryptamine; ACh, acetylcholine.

ences). The actions of phenoxybenzamine and imipramine suggest that under normal laboratory conditions there is a continuous release of NA. (If NA has a transmitter function in heat loss pathways in cats, then the effect of imipramine should be greater when these pathways are strongly activated, as in a hot environment. Such experiments do not seem to have been made.) Evidence for believing NA has a physiological role in activating heat loss pathways was recently reported by Myers and Chinn (150). With tracer techniques and "push-pull" microperfusion of discrete hypothalamic sites, the release of NA was greatly increased when the cat was exposed to a temperature of 40°C but not of 10°C. The sites where this release was seen were only in the anterior hypothalamus between the anterior commissure and the optic chiasma and within 1.5 mm of the midline.

Responses to 5-HT have not been as consistent as those to NA (table 1). Various authors report hyperthermic, isothermic or hypothermic effects depending on the dose used, but the necessary systematic study of the dose-response relationship has not been made. There is evidence for a release of endogenous 5-HT in perfusates of the third ventricle (81), but this release has not been tested with thermal stimuli as has been done in the case of monkeys (146). Thus there is still uncertainty

about the action of exogenous 5-HT in the cat's heat conservation pathways.

ACh is an obvious transmitter candidate in hypothalamic pathways although it does not seem to be present in particularly high concentrations. The evidence summarized in table 1 indicates the presence of "nicotinic" receptors in heat loss pathways and "muscarinic" receptors in heat conservation pathways. Microinjection experiments (176) have shown that cholinoceptive sites are widely scattered between the levels of the optic chiasma and the mammillary bodies, in contrast to the restricted sites in the PO/AH area for NA and 5-HT.

The general area from which temperature effects can be elicited by microinjections of drugs coincides with the area in which temperature-responsive neurons have been found. The region close to the midline and extending from preoptic level to the level of the mammillary bodies has been found to contain neurons which show a high sensitivity (in terms of discharge rate) to brain temperature. The technique of microiontophoresis has been used in an attempt to establish a more direct correlation between drug and temperature responses at the single cell level. Single hypothalamic neurons were first tested for their sensitivity to small imposed changes in hypothalamic temperature and then to the local application of NA, 5-HT and ACh from a multibarrel electrode. On the pharmacological evidence it might be expected that warm-sensitive neurons would be excited by NA, cold-sensitive neurons excited by 5-HT and both types excited by ACh. The reverse inhibitory relation might also be predicted.

These predictions have not been substantiated. Beckman and Eisenman (19) found that NA had the opposite effect to the predicted one since it inhibited the activity of warm-sensitive neurons and excited cold-sensitive neurons. 5-HT and ACh were generally without action. They also reported that a group of cells with a particularly high temperature sensitivity and possibly acting as thermodetectors (70) were not affected by the drugs but were responsive to current from the electrode. If these were thermodetectors then they might be expected to lack a synaptic input. Jell (124) could not confirm the work of Beckman and Eisenman, but reported "a total lack of correlation between thermal response pattern and drug sensitivity." Jell's conclusion has been confirmed (D.M. Ford, unpublished) by observations made in an isolated preparation of the cat hypothalamus (91).

The lack of correlation seen in the iontophoretic experiments could be explained if NA and 5-HT are not involved in integrative circuits in the cat hypothalamus nor with the efferent pathways leading from these. Instead these two monoamines may be functioning in afferent inputs to the hypothalamus, originating possibly from temperature sensors in the skin and other parts of the body. The fluorescent histochemical evidence for this view has already been reviewed (section III B). The cell bodies containing 5-HT are concentrated in the brain stem raphé nuclei and evidence has recently been provided (6) for a functional connection from there to the tissue in the walls of the third ventricle: electrical stimulation of the raphé causes a release of 5-HT into a perfusate of the third ventricle. This connection may not of course represent a thermal afferent pathway, but there is some anatomical evidence that the spinal ventrolateral tract, which carries afferent temperature fibres, forms one of the inputs to the raphé nuclei (3). The cell bodies containing NA with ascending fibres to the hypothalamus lie in the ventromedial region of the mesencephalon. There are now indications that both this region and the raphé have a direct or indirect link with neurons sensitive to local temperature in the PO/AH area. Eisenman (69) has found that most of these neurons were excited or depressed when the NA or 5-HT cell body regions of

the midbrain were electrically stimulated. But since according to the iontophoretic evidence the thermosensitive neurons themselves do not have receptors for the monoamines, there must be interneurons with a different transmitter substance between the ascending monoamine fibres and the thermosensitive neurons.

The best evidence for a transmitter function among the candidates is that relating to NA. This amine has a consistent hypothermic action and it has been shown to be released, as predicted, from highly localized hypothalamic sites during external warming (150). 5-HT does not seem to have a reproducible hyperthermic action which would be consistent with a putative transmitter action so that the original "balanced release" hypothesis of Feldberg and Myers (79) has not been fully substantiated. The position of ACh is that it may have a role in both heat loss and heat conservation pathways.

2. Dogs. Dogs respond in the same way as cats to intraventricular injections of the two monoamines NA and 5-HT (74, 75). 5-HT initiates shivering and there is a rise in body temperature, but there does not seem to be the prolonged hyperthermia found in cats. NA (or adrenaline) has the opposite effect and causes a rapid reduction in body temperature due to vasodilatation and probably lowered heat production. In dogs which were shivering during recovery from pentobarbitone anaesthesia, there was an abrupt cessation of shivering after an ICV injection of catecholamine. However, not all the evidence indicates a consistent hypothermic effect with NA (66). As was the case with cats, there has been an attempt to correlate the sensitivity of individual hypothalamic neurons to the monoamines with their sensitivity to local changes in brain temperature. Again as in cats, no such correlation was found (57). Both 5-HT and adrenaline depressed the firing rate of neurons irrespective of whether or not they were temperature sensitive.

ACh or cholinomimetics do not appear to have been used in dogs.

3. Primates. Injections into the third ventricle of anaesthetized rhesus monkeys established that they responded to NA and 5-HT in the same manner as cats and dogs (74). NA produced hypothermia by suppressing shivering and causing skin vasodilatation, while 5-HT had the reverse action. In addition 5-hydroxytryptophan, the precursor of 5-HT, was found to have the same action as 5-HT itself.

The hypothermic action of NA was confirmed in conscious baboons by Toivola and Gale (190) but these workers did not observe any hyperthermia with 5-HT. The dose level would seem to be critical since Myers (145) found a temperature rise with small doses of 5-HT in monkeys but a fall with larger doses.

A more precise localization of the active sites for NA, 5-HT and ACh has come from a systematic exploration of the hypothalamus and brain stem in conscious monkeys with microinjections of these drugs (160) in volumes of 1 μl or less. 5-HT and NA were found to have their respective hyper- or hypothermic actions only in the PO/AH region at the level of the anterior commissure. More caudal injections were without effect. In contrast, the sites at which ACh affected body temperature were more widespread, extending from the preoptic region back to the level of the mammillary bodies. At most of these sites, ACh caused intense shivering with a short-lasting hyperthermia. However at one circumscribed position in the posterior hypothalamus, close to the mammillary bodies, ACh microinjections produced a fall in body temperature which lasted for several hours.

The results of the microinjection experiments in monkeys have been summarized (160) in the form shown in figure 2. One difficulty with the proposed model is that cell bodies containing 5-HT or NA have not been observed in the anterior region by fluorescence microscopy. But even if

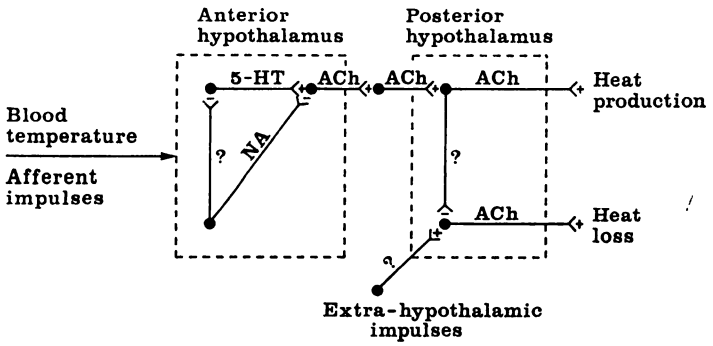

FIG. 2. Synaptic model based on microinjection experiments in monkeys. The abbreviations used are: 5-HT, 5-hydroxytryptamine; NA, noradrenaline; ACh, acetylcholine. (Redrawn from R. D. Myers and T. L. Yaksh: Control of body temperature in the unanaesthetized monkey by cholinergic and aminergic systems in the hypothalamus. J. Physiol. (London) **202**: 483–500, 1969.)

such cell bodies are not actually present, the model is not necessarily invalidated, because the anterior region could contain neurons which had receptors for 5-HT and NA but whose own transmitter was ACh.

The pharmacological results have been shown to have physiological significance in further experiments from Myers's laboratory. Two techniques were used to demonstrate the release of substances from monkeys exposed to hot or cold environments: a) cross-perfusion from the ventricular system of an exposed donor monkey to the ventricular system of a recipient monkey; and b) the assay of microperfusions from the hypothalamus.

The first experiments (144) showed remarkable results. If a donor monkey was heated and cerebrospinal fluid (CSF) from its third ventricle transferred to the third ventricle of a recipient monkey in a neutral environment, the temperature of the recipient monkey fell. Microperfusions of very limited hypothalamic regions confirmed these results (153).

These cross-perfusion experiments demonstrate directly that the release of factors may be enhanced or suppressed by external temperature changes, but of course the nature of the factors remained unknown. Bioassays of push-pull perfusions for 5-HT (146) and ACh (158) have been made. 5-HT (identified by biological assay and the use of specific antagonists) was released only during external cooling and only from sites in the PO/AH: these sites and the sites of sensitivity to microinjections of exogenous 5-HT coincided. More complex results were obtained from the assay of ACh during similar experiments. In the PO/AH region, ACh release tended to be enhanced by external cooling and suppressed by warming. This result corresponds to the hyperthermic action of ACh when administered by microinjection (fig. 2) into the PO/AH region. More caudal microinjections produced mixed results which corresponded to some extent with the hyper- and hypothermic actions of exogenous ACh (fig. 2). There have been no comparable assays for NA release in the monkey, but a thermally-induced release of this amine has been seen in the cat as already mentioned.

By virtue of the extent of the microinjection and microperfusion studies the monkey is the most thoroughly investigated of all the species in which putative transmitter function in temperature pathways have been considered. The results which have been obtained are entirely consistent with the idea that NA and 5-HT serve as transmitter substances in ascending, possibly thermal afferent, fibres which end in the PO/AH area. ACh, on the other hand, functions between interneurons in the black box.

Understandably, little experimental

work has been done on man although it might be expected that a reaction similar to other primates would be shown. As early as 1936, ACh was injected into the lateral ventricle of eight patients (111). Sweating was sometimes seen and in one case it was sufficient to lower body temperature. Eserine had a similar action, suggesting that the exogenous ACh was mimicking the effect of naturally-released ACh.

Phenylketonuric children, who would be expected to have a low level of brain 5-HT, have been studied: by analogy with monkeys (146) this deficiency might be expected to interfere with heat conservation responses to a cold environment. However just the reverse has been found (22). Compared with normal children, those with phenylketonuria showed undisturbed responses to cold, but in the heat their sweating was diminished. Whether this finding can be related to brain amine levels is dubious in view of the widespread nature of the metabolic lesion.

4. Sheep. Cats, dogs and primates all give reasonably similar body temperature responses to ICV injection of drugs. Quite different and more variable responses have been reported in other species such as sheep, rabbits and rats. Bligh and his associates have made extensive use of sheep and in particular they have produced a neuronal model based on the interaction between ICV injections of putative transmitter substances and ambient temperature. This model is shown in figure 3 and it provides an explanation of where the drugs may act in relation to the peripheral and hypothalamic temperature sensors. To date all the predictions to which this model gives rise have been validated. The evidence on which the model is based will now be discussed.

The first experiments (24, 174) were done at "room temperature" and suggested that 5-HT promoted heat loss with a consequent fall in body temperature while NA had the reverse effect. These are opposite effects to those generally seen when these substances are given to cats, dogs and monkeys. It was proposed (25) that an explanation for these species differences might lie with the prevailing ambient temperature. If any of the substances injected into the ventricles had an inhibitory action, that substance would only act on thermoregulatory pathways which were already active. Similarly an excitatory substance would only act on a pathway which was not already fully activated. The extent to which the various effector components making up the control action part of figure 1 will be active at a given ambient temperature will vary between species. For example a monkey, having thin fur, will show pronounced shivering at a higher air temperature than a well-insulated sheep. Thus a drug which activated heat production might have little action in a monkey already shivering but in a nonshivering sheep at the same temperature the same drug could promote vigorous shivering.

In the experiments of Bligh *et al.* (26)

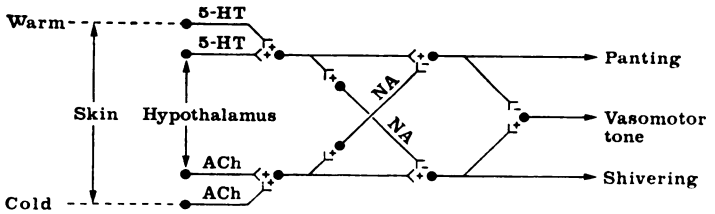

FIG. 3. Synaptic model based on intracerebro-ventricular injections in sheep. The abbreviations used are: 5-HT, 5-hydroxytryptamine; NA, noradrenaline; ACh, acetylcholine. (Redrawn from J. Bligh, W. H. Cottle and M. Maskrey: Influence of ambient temperature on the thermoregulatory responses to 5-hydroxytryptamine, noradrenaline and acetylcholine injected into the lateral cerebral ventricles of sheep, goats and rabbits. J. Physiol. (London) **212**: 377–392, 1971.)

sheep were exposed to a variety of air temperatures and NA, 5-HT and ACh or carbachol were given by ICV injection. NA had a general inhibitory action on whatever thermoregulatory activity was present at a particular temperature. At high air temperatures panting frequency was reduced and body temperature rose; in the cold, shivering was suppressed so that body temperature fell. The possible role of NA is shown in the model (fig. 3) as a transmitter in the crossed inhibitory pathways. Very similar effects were found with the sympathomimetic drug, clonidine (138).

5-HT activated those heat loss mechanisms not already activated by the environment and suppressed any heat production activity. For example, skin vasodilatation and panting appeared at intermediate air temperatures and shivering was suppressed in the cold. Consequently the effect of 5-HT was hypothermic except in the heat when heat loss mechanisms were already fully deployed. The upper pathway in figure 3 illustrates how 5-HT may have these excitatory actions with a noradrenergic inhibitory link.

ACh appears to function in a converse way to 5-HT, having an excitatory action on pathways leading to heat production and an inhibitory effect on any heat loss activity. Thus, as shown in figure 3, ACh has a hyperthermic action in both warm and cold environments. The receptors seem to be of the muscarinic type (125).

The interaction between ICV injections and the ambient temperature presumably implicates a thermal input from the skin. The model in figure 3 indicates a convergence between inputs from skin and hypothalamic thermal sensors with both inputs using the same transmitters. ICV injections of NA, 5-HT and carbachol have just the predicted effects if given when the hypothalamic sensors are activated by localized warming and cooling (137). For example during hypothalamic cooling, which activated shivering, an ICV injection of carbachol increased the intensity of the shivering; 5-HT and NA abolished the shivering.

It is remarkable that the consistent results which led to the formulation of the model of figure 3 were obtained from injections into a lateral ventricle. Presumably the substances were acting through the walls of the third ventricle on hypothalamic structures. So far, more precise sites of action have not been defined.

The concept that ambient temperature has an influence on the thermoregulatory effects of drugs and might explain species differences has not been widely tested on animals other than sheep. The general inhibitory action of NA does not seem to apply to cats. At both low and high ambient temperatures there was a fall in body temperature with NA rather than the reversal which would be predicted from the model in figure 3 (Feldberg and Hellon, unpublished results).

5. Goats. Goats respond to ICV injections of the monoamines in a very similar way to sheep. Andersson *et al.* (5) observed that 5-HT given at an air temperature of 18 to 20°C caused hypothermia due to vasodilatation and panting. The responses of goats at different air temperatures (26) can be adequately represented by the diagram in figure 3 which is based on data from sheep.

6. Oxen. Oxen have not been studied extensively but they were indeed the first animals in which an interaction between the effect of ICV monoamine injections and air temperatures was shown (88, 89). NA given at 30°C air temperature had no action, but at −1°C shivering was stopped and oxygen consumption fell as did body temperature. This action is similar to that in sheep and goats. However 5-HT also had a hypothermic action but only in warm environments. ACh had no action.

Thus oxen do not appear to possess opposing responses to two different substances as, for example, sheep do to 5-HT (hypothermic) and ACh (hyperthermic).

7. Rabbits. Rabbits were the first species

shown to have body temperature responses to ICV injections of NA and 5-HT which were different from the original findings of Feldberg and Myers in cats (79). In 1965 two laboratories reported that rabbits reacted in the opposite way to cats, with NA having a hyperthermic action and 5-HT being weakly hypothermic (43, 174). These and all the other findings on rabbits are summarized in table 2. All the pharmacological evidence points to the involvement of NA in pathways controlling heat production and conservation in rabbits; 5-HT activates heat loss and so apparently does ACh.

As in other species there are neurons in the hypothalamus (37, 108) and midbrain (163) of the rabbit which are highly thermosensitive. These specialized neurons have been discussed in section II and the possibility that they may be specifically acted upon by intraventricular drug injections has been examined in cats and the results were negative. In rabbits however, when NA, 5-HT and ACh were applied to single thermoresponsive neurons from multibarrelled micropipettes, the precise effects which might have been predicted from ICV injections were found (118). Warm-sensitive neurons were excited by 5-HT and/or depressed by NA. Cold-sensitive neurons were excited by NA and/or depressed by 5-HT. ACh was not effective nor was there any particular pattern in the drug responses of thermally insensitive neurons. It is not yet clear why these positive results should have been found in rabbits in the face of negative results in

TABLE 2

Summary of data from rabbits

Drug	Technique	Ambient Temperature or State of Animal	Effect on Control Actions	Effect on Body Temperature	Remarks	References
NA*	ICV NA		Vasoconstriction	↑1–3°C		43, 174, 199
	ICV NA			↑	Graded dose effects	123
	ICV reserpine		Vasoconstriction	↑2°C	Later doses ineffective	13
	ICV desipramine	19–21°C		↑	Not after NA depletion	49
	ICV NA	Hot	Polypnea suppressed	↑1°C		26
		Cold	Shivering suppressed	↓0.5°C		
	ICV desipramine	8–29°C		↑	Hyperthermia increased with ambient temperature	67
	ICV phenoxybenzamine	19–22°C	Vasodilatation, polypnea	↓1.2–2.3°C	Not after NA depletion	63, 86
	ICV phenoxybenzamine with abdominal cooling		Normal control actions blocked	↓		169
	ICV 6-hydroxydopamine			↑1.6°C	Weaker response to second dose	130a
5-HT	ICV 5-HT			↓0.4°C		43, 174
	ICV 5-HT 10–30 μg			↓	Reversal with high doses	123
	≥100 μg			↑ Dose-related		
	ICV 5-HT	Hot		↓0.4°C		26
		Cold	Shivering suppressed	↓0.4°C		
	ICV or intracisternal 5-HT or 5-HTP		Ear vasodilatation, polypnea	↓1.5°C		14
	ICV tranylcypromine			↓0.3–0.6°C		55
ACh	ICV ACh and eserine or carbachol	Hot	Panting, vasodilatation	↓0.3°C	Weak effects	26
		Cold	Shivering suppressed vasodilatation	↓0.6°C		

* The abbreviations used are: NA, noradrenaline; IVC, intracerebro-ventricular; 5-HT, 5-hydroxytryptamine; ACh, acetylcholine; 5-HTP, 5-hydroxytryptophan.

cats reported from three laboratories [19, 124, D. M. Ford (unpublished results)]. Confirmation of the findings in rabbits is awaited with interest.

8. Rats. The results of experiments on the central pharmacology of temperature regulation in rats add *intra*-species confusion to the *inter*-species controversy. Summarized in table 3 are the findings from experiments in which ICV injections or microinjections have been used. The action of NA may be hyperthermic with small doses or hypothermic with larger doses. Presumably the first of these effects provides a better simulation of the natural release of NA. However, estimates of the NA content of nerve terminals in the hypothalamus of rats exposed to low temperatures (3°C) and high temperatures (40°C) would lead to the opposite conclusion: in heated rats there were signs of increased activity in NA terminals, but in cooled rats there was no change (48). Measurements of the turnover of NA in the hypothalamus also showed an increase in the heat, but a similar increase was found during cold exposure (183). This dual response to heat and cold, which was confined to the hypothalamus, would be in keeping with the role proposed for NA by Bligh (23) as a crossed inhibitory transmitter between heat conservation and heat loss pathways (*cf.* fig. 3). This possibility could be tested by making ICV injections of adrenergic blocking agents under hot and cold conditions.

The content of 5-HT in nerve endings is reduced in rats which have been exposed

TABLE 3

Summary of data from rats

Drug	Technique	Ambient Temperature or State of Animal	Effect on Control Actions	Effect on Body Temperature	Remarks	References
NA*	ICV NA	21–23°C		↑ <6 μg ↓ >10 μg	Dose reversal	76
	ICV NA	22–24°C		↑	Dose dependent	159
	Microinjection of NA	25°C		↑	Dose dependent	17
		−8°C	Rats switched on heat lamps		20–150 ng	
	Microinjection of NA	24°C		↓		9
		5°C		↑		
	ICV or intracisternal 6-hydroxydopamine	22–25°C		↓	Dose dependent Abolished by 6-OHDA pretreatment	30, 162, 185
5-HT	ICV 5-HT	21–23°C		↓	?Dose dependent	76
	ICV 5-HT	22–24°C		↓	Dose dependent	159
	Microinjection of 5-HT	7 or 23°C		↑	Dose dependent	56
	ICV 5-HT 10 μg	23°C	Tail vasodilatation	↓	Indicates extrahypothalamic site	56
ACh	ICV ACh and eserine	22–24°C		↑		159
	Microinjection of carbachol	5 or 23°C		↑		8, 9
	Microinjection of ACh	−5°C	Reduced switching on heat lamps	↓	Dose dependent	18
	Implantation of carbachol	24°C		↓2°C		119
	Iontophoresis of ACh	22°C		↓0.9°C		128
	Microinjection of pilocarpine	22°C		↓1.3°C		132
	ICV carbachol	23°C	Marked depression of O_2 consumption	↓	Dose dependent	139

* The abbreviations used are: NA, noradrenaline; IVC, intracerebro-ventricular; 5-HT, 5-hydroxytryptamine; ACh, acetylcholine; 6-OHDA, 6-hydroxydopamine.

to heat and increased in those exposed to cold (48), thereby suggesting that 5-HT-containing neurons may be concerned with activating the processes of heat loss from the body. In addition, an increased turnover of hypothalamic 5-HT has been observed in heat-exposed rats (184). Thus it might be expected that ICV injections of 5-HT would lead to hypothermia in rats: indeed that has been found (table 3).

Other evidence for an action of 5-HT on the PO/AH area of rats comes from Aghajanian's laboratory. The chief group of cerebral neurons showing a specific fluorescence for 5-HT have their perikarya in the midbrain raphé nuclei and nerve endings in the hypothalamus and spinal cord (4). Electrical stimulation of the raphé nuclei in rats caused a rise in body temperature and at the same time there was an increase in the forebrain content of the 5-HT metabolite, 5-hydroxyindole acetic acid (181, 182). Exposure of rats to heat also increased the level of 5-hydroxyindole acetic acid in the forebrain which was prevented if lesions had been made in the raphé nuclei (201). It has also been shown that raphé neurons (presumably containing 5-HT) are excited when the rat's temperature is raised by infra-red radiation. Thus we have the apparent paradox of an ascending (afferent) system which, when activated by external heating releases 5-HT, and when activated electrically also releases 5-HT but *raises* body temperature. This amounts to an apparent positive feed-back situation and needs further study.

There is total disagreement about the thermal effects of the intracerebral administration of ACh or cholinomimetics (table 3). In the rostral hypothalamus, neurons have been described which were excited by warming the tail and also excited by local or systemic infusions of ACh or nicotine (129). It is uncertain what role these neurons have in the black box.

Attempts have been made in rats to correlate the drug sensitivity of single hypothalamic neurons with their sensitivity to local (hypothalamic) thermal stimulation. Beckman and Eisenman (19) found that both NA and 5-HT tended to suppress the activity of warm-sensitive neurons in the PO/AH area. Murakami (142) tested warm- and cold-sensitive neurons with NA, 5-HT and ACh, but failed to find any regular association between drug and thermal sensitivities.

9. Mice. In mice, both NA and 5-HT given by the ICV route, cause a dose-dependent fall in body temperature (32). There is evidence that the hypothermia caused by NA at normal temperatures can be reversed to hyperthermia if the amine is given at room temperature of 35°C (103). This finding is reminiscent of the similar finding with NA in sheep and goats as represented in the model shown in figure 3.

The change in the content of NA and 5-HT in the whole brain of mice has been measured after exposure to high and low temperatures (106). There was a decrease in the NA content after 1 hour of exposure to 40°C and in the cold the 5-HT content tended to be diminished. It is difficult to know what interpretation to place on these findings.

Although the hypothalamus has the highest concentration of histamine in the brain, the possibility that histamine might play some (unspecified) part in the hypothalamic control of temperature has barely been tested. In mice a graded dose-dependent fall in temperature has been found when between 1 and 10 μg of histamine is given into a lateral ventricle (180). The experiments were done at an unspecified room temperature and their significance has yet to be determined. When histamine was infused into the hypothalamus of a rabbit, no effect was seen (43).

10. Guinea pigs. Work on thermoregulatory mechanisms in guinea pigs differs from species which have been considered so far because attention has been directed towards the activation of brown fat me-

tabolism. The brown fat is concentrated mainly in a pad between the scapulae and is concerned in the generation of heat especially in young animals. The hypothalamic mechanisms controlling brown fat metabolism have been carefully studied by Brück and his colleagues, who have shown that NA and ACh are involved.

Microinjection of NA into the medial hypothalamus has been found to activate the brown fat and so raise body temperature (207). The injection site was just caudal to the level of the anterior commissure. Cold-induced activation of brown fat could be inhibited by localized warming of the hypothalamus, but at a separate more rostral site just in front of the anterior commissure. At this thermally sensitive site, the inhibitory effect of local warming could be mimicked by microinjection of ACh or carbachol. The receptors concerned appear to be nicotinic (208).

Thus there are separate sites in the guinea pig for the induction of brown fat activity by NA and the inhibition of this activity by local warming.

11. Hibernators. The nature of the radical changes in normal temperature regulation which occur when an animal enters hibernation are still the subject of lively debate (23). Two possible mechanisms which might explain why body temperature can fall to less than 10°C are: (a) that the set-point is somehow lowered to that level and an "impaired" type of temperature regulation continues during hibernation; and (b) that regulation of temperature is "switched off" during the hibernating state and body temperature follows passively the environmental temperature.

The possible involvement of NA and 5-HT in the cerebral control of hibernation is suggested by two groups of observations. In hedgehogs the whole brain content of 5-HT rises sharply during hibernation and at the same time the NA content decreases (191, 192). As with all whole brain measurements it is not readily apparent how these changes can be related to just one of the many functions which are controlled from only a small fraction of the whole brain.

More direct evidence has been provided by intrahypothalamic injections into ground squirrels during hibernation (20). Both NA (0.4 μg) and 5-HT (o.8 μg) aroused the squirrels from hibernation and the rate of rise of body temperature was the same with both the amines as it was with a spontaneous arousal. Injections had this effect in the PO/AH region only. When similar injections were made into nonhibernating squirrels, both drugs also raised body temperature. Whether the arousal effect with the drugs does in fact mimic natural events must await further observations.

12. Exotica. The echidna (*Tachyglossus aculeatus*) is a monotreme which has evolved quite separately from the placental mammals and the marsupials. When the echidna's responses to ICV injections were observed (11) it was found that 5-HT, NA and cholinomimetics all caused a fall in body temperature irrespective of whether the ambient temperature was cool, neutral or warm. These findings present yet another pattern of responses to these drugs.

One member of the *Camelidae,* the alpaca, has been tested (16) but it failed to show any changes in body temperature after ICV injections of the monomines or a cholinomimetic.

D. Discussion

The literature which has just been surveyed raises many questions about the function and significance of the monoamines and ACh in the regulation of body temperature by the hypothalamus. There are two points of major importance which need to be considered in some detail: a) What explanations can be offered for the wide differences in the reactions of different species? b) How far can the monoamines and ACh be considered as actual

neurotransmitters in thermoregulatory pathways?

1. Species differences. There are probably multiple explanations for the bewildering catalogue of effects which has just been related for the various species. Among the possible reasons which can be advanced are the following:

a) The variation between species may be genuine. This implies that for the control of a given function, such as shivering, various animals use different central transmitters. A close look at the evidence reveals that it is very difficult to find an unequivocal example of a real species difference. The reasons lie in the multiplicity of techniques which have been used and these will be mentioned below. Perhaps the best documented difference is between the responses to ICV injections of NA in cats, dogs and monkeys which show hypothermia and in sheep, goats and rabbits which show hyperthermia. But even in this instance there is uncertainty as to the modulation of NA effects by the ambient temperature.

b) If the ICV or intrahypothalamic injection of a substance is to simulate the function of a naturally-released transmitter, then the dose will certainly be critical. With increasing doses, initial excitatory effects may be followed by synaptic block if a sufficiently high concentration is achieved at the receptor sites.

Such a reversal of effect is well illustrated by the injection of NA into rats (34). Doses between 1 and 4 μg caused graded reductions in body temperature but higher doses up to 32 μg had progressively less action. Similarly with 5-HT in rabbits the initial fall in temperature produced by the injection of 10 or 30 μg becomes a rise with doses of 200 μg or more (123). Such examples serve to emphasise the importance of establishing a proper dose-response relation for each substance and each species. The failure to do so, and such failure is evident in some of the literature, could be a contributory factor in actually creating apparent species differences.

c) There are three anatomical features which might contribute to the apparent variation between the species. Firstly, the neuroanatomy of the thermoregulatory pathways is not known in any detail. It is quite possible that some species might have synapses in the pathways which are closer to the ventricular walls than in other species and thus more accessible to drugs introduced into the ventricular system. Even if such variation does not occur, there will still be differences in accessibility to ICV drugs due to differing brain sizes. For example in a rat probably all the synapses in the PO/AH area relating to temperature control are to be found within a distance of 2 mm from the ependyma of the third ventricle. In a larger brain, such as in a sheep or monkey, this distance will be of the order of 4 or 5 mm. Hence the possibility of drugs diffusing out from the third ventricle to reach a given group of synapses is bound to vary with brain size.

A second factor which could be important in determining the response to an ICV injection, and which has received almost no consideration, is the volume used particularly in relation to the ventricular volume of the species. It is generally assumed that drugs put into one of the lateral ventricles have their action on the PO/AH area after diffusing through the ependyma of the third ventricle. In some cases results confirm that this assumption is likely to be correct. For example in cats the temperature responses are similar when injections are made intraventricularly or directly in the PO/AH (79, 80). However in rats, 5-HT was found to give opposite effects depending on whether ICV injections or microinjections were used (56). It was assumed that the ICV effect was produced from a more caudal site which might be reached from the aqueduct, the fourth ventricle or even subarachnoid space. In some species like rats it is known that volumes of 5 μl spread

within 10 seconds from a lateral ventricle to the fourth ventricle (159), so that distant or multiple sites for drug action can occur. Unfortunately there seems to be no available data on ventricular volumes in various species, so that matching of volume injected to the ventricular volume could only be made by trial and error.

A third anatomical variable is that of body size. Apart from the obvious question of drug dosage, body size will clearly affect the heat capacity of a species and also its surface area:mass ratio. To take the extreme examples from our literature, the mice used by Brittain and Handley (32) weighted 20 to 24 g with a calculated area:mass ratio of 5.2 cm^2/g; the oxen of Findlay and Thompson (89) weighed 150 to 270 kg with a corresponding ratio of 0.17 cm^2/g. Between those two species the weights vary by a factor of 10^4 and the area:mass ratios by a factor of 30. If, as has often been the case, rectal temperature is used as the only index of thermoregulatory reaction during the intracerebral injection of drugs, then in a larger animal no temperature change might be seen if the drug only caused, say, a transient vasodilatation of the skin blood vessels. On the other hand, in a mouse such a vasodiltation could produce a profound drop in rectal temperature.

d) The importance of the ambient temperature in which the experiments a made has already been discussed in relation to sheep. Bligh *et al.* (26) have shown how the action of NA can be reversed by changing the external temperature (see fig. 3). This concept was also suggested as at least a partial explanation for the species differences. It was argued that NA, being inhibitory, would only act on the pathway which was already being driven by the ambient temperature. In the cold, for example, NA in sheep and goats suppresses shivering and consequently body temperature falls. Now the ambient temperature required to produce shivering will vary between species and thus at any particular ambient temperature the effect of NA might vary between species.

In fact, this idea has not yet been adequately tested in species other than those from which it originated—sheep and goats. Some unpublished observations on cats (Feldberg and Hellon) and a short report on rabbits (67) indicate that in these species the action of NA on body temperature is not reversed by changing the ambient temperature from low to high.

2. Transmitter function. How far can the evidence which has been surveyed be said to establish that 5-HT, NA and ACh are acting as neurotransmitters between hypothalamic neurons concerned with thermoregulation? On the available evidence none of these substances satisfies the strict 6-fold criteria for neurotransmitters of McLennan (133) or even the simplified criteria of Werman (203). Several attempts have been made, with microiontophoresis, to correlate the drug sensitivity of hypothalamic cells with their responses to local temperature. Most studies of this type (19, 124) have failed to establish any such correlation. Only Hori and Nakayama (118) have been able to relate drug sensitivity with thermal sensitivity of single neurons in rabbits on the pattern which might have been expected from the effects of ICV injection in that species.

Perhaps the best positive evidence of transmitter function has come from the experiments in Myers's laboratory in which perfusate from the hypothalamus of a heat-stressed or a cold-stressed monkey was passed into the same brain area of an unstressed monkey. The recipient responded in the same way as the donor, suggesting that some specific substances were being transferred (153). In later experiments, 5-HT, NA and ACh were shown to be released from specific sites in the hypothalamus and brain stem of monkeys and cats in response to external temperature changes (146, 150, 158). Furthermore, the pattern of release was related to the ambient temperature in a way which would

have been predicted from the animals responses to ICV injections. For example, 5-HT raises temperature in monkeys by causing vasoconstriction and shivering; when these same mechanisms are activated by external cooling then 5-HT is released. Clearly more of these elegant experiments are needed since, besides providing valuable evidence for transmitter function, they may also help to explain the mystery of species differences.

E. Conclusions

What significance can be assigned to the evidence which has been reviewed? How far has this work contributed to an understanding of the control system for body temperature depicted as a physical analogue in figure 1? Has the functioning of the black box become any clearer? It could be argued that far from providing a better understanding of these matters, this work on neurotransmitters has tended to make the situation more obscure. Nevertheless it is still possible to present an hypothesis which provides a rational explanation for the functioning of NA, 5-HT and ACh as candidate neurotransmitters in brain stem pathways controlling body temperature. The hypothesis, which was tentatively proposed by this reviewer in 1968 (109), is that the monoamines serve as neurotransmitters in afferent pathways which relay information from thermoreceptors in the skin and elsewhere to the hypothalamus and midbrain; ACh acts in integrating pathways in the hypothalamus. The evidence for these views is scattered in the preceding pages and may now be gathered together.

a) Fluorescence histochemistry (section III B) shows that NA and 5-HT are confined almost entirely to nerve endings in the hypothalamus. The cell bodies are found more caudally in the raphé nuclei for 5-HT and in ventral areas of the brain stem for NA. Therefore the monoamines must be associated with an input to the black box of figure 1. b) Electrical stimulation of these cell body areas has been found to excite PO/AH neurons which are sensitive to hypothalamic temperature (69). Thus there is a specific link, which need not be monosynaptic, between the monoaminergic neurons and more rostral neurons which can be presumed to be part of the controlling system. c) External heating can excite the firing rate of neurons in the raphé nuclei (201). In addition one of the inputs to these nuclei comes from the anterolateral spinal tract which carries the thermal afferent fibres (33). d) External heating and cooling can also cause the release of NA and 5-HT respectively from localized sites in the PO/AH (146, 150, 158).

All this evidence points to NA and 5-HT serving as neurotransmitters for neurons carrying thermal information to the black box. It is possible that one amine is associated with the skin and deep body "warm" sensors and the other with the "cold" sensors. If this suggestion is confirmed, it would explain why it is possible to obtain a co-ordinated series of control actions from such a crude technique as the injection of drugs into the cerebral ventricles. The amines would be diffusing through the ependyma of the third ventricle and simulating the mass discharge of either the warm or the cold peripheral thermosensors.

We now need much more evidence to test this hypothesis. The combined techniques of neuropharmacology, neurophysiology and neuroanatomy will have to be used and, ideally, a complete set of data should be gathered for each of the common laboratory species. At the moment the evidence in favour of the hypothesis has had to be collated from experiments on a variety of animals.

IV. Fever, Pyrogens and Antipyretics

The fevers associated with infection, antigen-antibody reaction or necrosis are one of the commonest disturbances of the temperature regulatory system. In the present

context the reason for discussing fever is the light it can shed on the functioning and pharmacology of that system. The aspects of fever which will be considered include the nature of pyrogens, their site and mode of action, the involvement of prostaglandins, and the action of antipyretics. Publications appearing in the last few years have greatly increased our understanding of all these aspects. The generally accepted view of the processes involved in generating a fever is now as follows. Invading organisms react with cells of the host causing the release of a pyrogenic substance. The latter acts on the temperature-sensitive neurons in the PO/AH area to change their temperature/activity relationship. The output from these neurons passes to the periphery where the control actions for heat loss are suppressed and those for heat conservation are activated. Shivering is the commonly observed sign of a fever but in species possessing brown adipose tissue this may also be a source of heat in fever (188). Prostaglandins of the E series appear to be synthesized in the brain during a fever and to be concerned in pyrexia; antipyretics probably act by inhibiting this synthesis.

In welcome contrast to the variable and conflicting results which have so far come from the work on the monoamines and other drugs, the fever response to pyrogens is very similar in almost all species. Exceptions which have been reported are rats,* mice and guinea pigs (194).

Among the many external agents which are capable of producing fever on systemic injection are Gram-negative and Gram-positive bacteria, mycobacteria, viruses, the 5 β-steroids (29a) and various colloids. These will be classified as exogenous pyrogens (ExP). Pyrogenic substances produced or released in the body by the action of ExP will be called endogenous pyrogens (EnP).

A. Composition of pyrogens

As just indicated, a wide variety of agents can give rise to a fever, but most experimental work has been done on the ExP which can be extracted from the cell walls of Gram-negative bacteria. This ExP is a lipopolysaccharide of high molecular weight (about 10^6) and its detailed chemistry has been reviewed recently (205). It is not certain whether an ExP can act directly to cause a fever, but most of the evidence suggests that this does not happen. During phagocytosis a final common effector substance (EnP) appears to be released, and it is this substance which actually has the pyrogenic action.

EnP can be derived from a variety of cells in the body including circulating leucocytes and monocytes, Kupfer cells in the liver and macrophages from the lungs, spleen and lymph nodes (7) and certain renal tumours (51). The release of EnP from these various cells depends upon their contact with an ExP. At present it is uncertain whether there is more than one type of EnP, but that derived from rabbit peritoneal exudate cells appears to be a small protein (143).

In addition to their chemical differences, ExP and EnP have different functional properties. As might be expected from the fact that ExP interacts with host tissues, fevers from ExP show a longer time to onset than fevers from EnP. Tolerance soon develops with repeated daily doses of ExP but not with daily EnP. Moreover, an animal made tolerant to ExP will still develop a fever with EnP. These aspects of pyrogen function have been reviewed recently (7, 54, 170) and need not be considered in detail here.

B. Site of action of pyrogens

Attempts to show that pyrogens have a direct action on peripheral structures have had negative results. Frens (96) was un-

*Recent evidence suggests that rats may indeed be sensitive to pyrogens provided the dose is suitably adjusted (188).

able to find any change in the properties of cold sensors in the tongue when pyrogens were given to rabbits, nor was a thermal reflex involving skin warm sensors affected in man (35). EnP does not have a direct vasoconstrictor action on blood vessels of the human hand (186). When EnP was given to a patient with complete transection of the spinal cord at the level of C6 (46), there was no vasoconstriction in the hand and only the innervated muscles shivered. The patient had intact spinal autonomic reflexes, so this evidence points to a site of action of EnP which is neither peripheral nor in the spinal cord below the level of C6. Direct injection of EnP into the subarachnoid space of the cord in rabbits also failed to elicit any fever response (173).

Indirect evidence for a cerebral site of action comes from experiments in which EnP was infused into a carotid artery (2, 127). The fever was greater and had a shorter latency than when the same dose of EnP was given into a vein. Bacterial ExP produced the same response by either route. In cats in which the hypothalamus had been destroyed, there was no fever response to ExP (15).

The direct introduction of pyrogens into the brain substance by microinjection has pointed strongly to the medial anterior hypothalamus being the target area. In cats (122) and rabbits (44, 173) the injection of EnP in 2 μl volumes into this region causes fever to develop with a latency of less than 10 minutes. Injections made slightly away from the medial PO/AH gave only an attenuated fever which was slow to develop. The anteromedial area is also the site of temperature-sensitive neurons and for the action of some of the monoamines. It is therefore possible that EnP acts on these neurons or through the monoamines, and these possibilities will be discussed below.

A fever can also be produced when ExP is microinjected into the PO/AH (152, 196), but it is not clear whether the effect involves the local production of EnP at the site of injection, perhaps by invading leucocytes. Certainly the latency of 1 hour or more to the beginning of the fever is much longer with an injection of ExP as compared to the latency of less than 10 minutes with EnP (44, 122).

Another site of EnP action has been reported in the brain stem reticular formation (173) from which a short-latency fever can also be elicited, but the response was somewhat slower and weaker than in the anterior hypothalamus.

Ideally, to establish the site of action pure EnP should be labelled and given to animals whose brains could then be autoradiographed to reveal sites of accumulation of the labelled molecule. With ^{131}I-labelled mixtures of serum proteins and EnP given by intracarotid injection, Allen (2) did in fact find an intrahypothalamic accumulation of radioactivity, but at a site which was more caudal than might have been expected on the basis of the microinjection of EnP.

Although the experimental evidence indicates the anterior hypothalamus as the sensitive target for EnP, it is not yet clear how circulating EnP, which is a small protein, can reach this brain area. Possibly the microcirculation is more permeable here but there is no good evidence for this. The possibility that EnP first escapes from the circulation into the CSF and then reaches its target by absorption from the third ventricle has not been supported (87).

C. Behaviour of hypothalamic neurons during fever

There have been several investigations which have shown that ExP, given systemically, can modify the properties of the temperature-responsive neurons in the PO/AH and there is an encouraging agreement among the findings. Warm-sensitive neurons (those whose activity is increased by brain warming) have their sensitivity depressed by pyrogen, while the cold-sensi-

tive neurons become more sensitive. This effect has been demonstrated in cats (68, 204) and rabbits (37). The same effect has also been found in a group of cold-sensitive neurons in the midbrain reticular formation (164). Only 13 temperature insensitive cells in the hypothalamus have been tested for sensitivity to pyrogens: none showed any change in activity. Pyrogens therefore appear to have a specific action on the temperature neurons in the PO/AH area and this action is that which might be expected if these particular neurons are concerned with the genesis of a fever. Such a selective action may be the ultimate mechanism of fevers, but two cautionary points need to be made: a) There is as yet no proof that the pyrogen action is directly or solely on these temperature neurons. b) The evidence is still insufficient to show unequivocally that pyrogens do not act on the temperature insensitive neurons. If techniques can be improved, it should be possible in the future to answer such questons by administering EnP onto individual neurons rather than by injecting ExP into the whole animal.

The consequence of the action of pyrogens on the thermoregulatory control system (fig. 1) is usually said to be an elevation of set-point. The available evidence is inadequate to allow any decision on whether a real change in set-point takes place (141). In any case, Cooper (42) has recently cast doubt on the relevance of the concept of a set-point to fever: ". . . is the term 'resetting the central mechanism at a new high level' a splendid example of a piece of jargon which hinders further inquiry and damps down research, or has it in fact any real value?".

D. Are the brain monoamines concerned with fever?

From the evidence that has been reviewed so far it is clear that pyrogen and the monoamines (NA, 5-HT) share the same site of action in the anterior hypothalamus. Thus an obvious question is whether the amines are concerned in the mechanism of fever. There is a general argument plus some experimental evidence against such an idea; there is also some direct evidence in favour of amine involvement in certain species.

1. Evidence against monoamine involvement. The complexity of thermal responses to cerebral injections of drugs has already been reviewed above and there is considerable uncertainty about the significance of the results, particularly the variations between species. Thus one argument (71) against an association between fever and the monoamines is the contrast between the action of pyrogens in most species and the highly variable action of the monoamines. For such an association to hold EnP would have to act on different species by releasing different monoamines. Some pharmacological evidence weighs against the involvement of the monoamines. The depletion of 5-HT or NA levels in the brain had no apparent effect on the development of a fever in rabbits (44, 60, 61). However negative results in depletion experiments are always difficult to interpret because the extent of depletion is variable and is never complete.

2. Evidence for monoamine involvement. The experiments of Teddy (189) seem to provide the best evidence that fever can be affected by depletion of brain NA or 5-HT. In rabbits NA given by ICV injection is hyperthermic; after NA depletion by α-methyl-*p*-tyrosine there was a significant reduction in the height and duration of the fever after EnP injection. Conversely, with the hypothermic amine 5-HT, depletion with *para*-chlorophenylalanine clearly led to an enhancement of fever. There are other results (135) confirming these findings with ExP rather than EnP injection.

The turnover of 5-HT, but not NA, in the hypothalamus of rats is increased in fever (136) but since the role of 5-HT in temperature regulation in the rat is equivocal (section III) the significance of this result is uncertain.

In terms of the role for the monoamines

suggested earlier (section III E) as transmitter substances in thermal afferent pathways, it might be that any monoamine involvement in fever would be as a consequence rather than as a cause. If, as all the evidence indicates, EnP acts centrally to cause a general skin vasoconstriction with the consequent lowering of skin temperature, then cold sensors would be activated and either NA or 5-HT released in the PO/AH. This release could have a reinforcing effect on the action of EnP. Present techniques for the microperfusion of specific brain sites would allow this idea to be tested.

E. Prostaglandins

The experimental study of fever was given a new impetus when it was discovered quite recently that prostaglandins (PG) of the E series play some part in the action of pyrogens on the central nervous system. It has been known for some time that prostaglandins of the E and F series are widely distributed in the brain without any particular region having an outstandingly high concentration (116). Subcellular fractionation of brain homogenates reveals that prostaglandins are present in several fractions including that of the nerve endings (117, 126). The release of PG or related substances has been reported from several cerebral sites, but no specific function had been suggested beyond an association with NA release; this will be discussed in below.

In 1971 Milton and Wendlandt (140) announced that injections of small doses of PGE_1 or E_2 into the third ventricle of cats and rabbits caused vigorous shivering, skin vasoconstriction and piloerection after a delay of only 1 or 2 minutes. Rectal temperature rose rapidly. The hyperthermia was dose-dependent between 10 ng and 10 μg. The other prostaglandins, (A_1, $F_{1\alpha}$, $F_{2\alpha}$,) had no immediate or strong action on temperature. The pyrogen actions of PGE_1 and E_2 have been confirmed in cats and rabbits and also been seen in rats and sheep (84, 99, 167). In fact the only species so far not reported to show hyperthermia in response to PG is a monotreme, the echidna (*Tachyglossus aculeatus*) (11).

The exact site of the hyperthermic action of prostaglandins is the PO/AH region close to the walls of the third ventricle (85, 187). The site in fact is indistinguishable from that at which pyrogens act, although of course at the cellular level there may be differences.

The hyperthermic action of PG is not a simple stimulation of heat production but is influenced by the ambient temperature. In rabbits and in sheep, it has been shown that under hot conditions the mechanisms for heat loss (evaporation and vasodilatation) were inhibited without increase in heat production (shivering); in the cold, PG acted by stimulating shivering (27, 187). Exactly the same sort of interaction has been seen in man when pyrogen was given under hot or cold conditions (165a).

This evidence suggesting that PG is concerned with the action of pyrogens was strengthened by the discovery that antipyretics, such as aspirin and paracetamol, have the property of inhibiting the synthesis of PG in brain and other tissues *in vitro* (90, 193). The inference that antipyretics might act by inhibiting the synthesis of PGE in the hypothalamus was clear.

Further evidence for involvement of PG was derived from the collection and assay of cerebrospinal fluid during a fever. This procedure has been carried out in cats with the third ventricle (72) and the cisterna magna (73) and the results were essentially the same in the two sites. The PG content of the CSF was very low before pyrogen was given. After a pyrogen was given there was a 10- to 20-fold increase in the PG content as determined by bioassay. That the PG was either E_1 or E_2 was established by thin-layer chromatography and now the actual substance appears on the basis of radioimmunoassay to be PGE_2 (59). When a cat with fever was given intraperitoneal injections of the an-

tipyretics, indomethacin, paracetamol or salicylate, its temperature fell and at the same time there was a sharp reduction of the concentration of PG in CSF to normal low values. This and other actions of antipyretics will be discussed in more detail below.

The discovery that PG is released into the ventricular space during a fever might also explain another interesting observation. If the ventricles are filled with an inert oil, fevers in rabbits are enhanced and prolonged (47). Presumably the oil prevents the normal passage of PG into the CSF.

A clear functional link has been established between pyrogens and prostaglandins as agents for causing a fever, but the details of their association, particularly at the cellular level have still to be worked out. Microinjections of EnP (44, 122) and PG (85, 187) into the same PO/AH region cause fever, but do they act on the same type of neuron? The only evidence so far obtained (92) about these questions indicates that when PGE_1 is applied to single neurons in the hypothalamus, it selectively excites those which also respond to brain cooling and does not affect neurons responding to brain warming or which are temperature insensitive.

The time taken for EnP injected directly into the hypothalamus to start a fever is about 7 minutes (44). This delay may be the time required to stimulate PG synthesis and release. A comparable injection of PG itself acts almost immediately (140, 187).

Does PG act through the monoamines? It is known that 5-HT perfused through the ventricles of an anaesthetized dog will release PGE (115). In addition PGE will inhibit the release or action of NA at various peripheral sites (107) and in the cerebellum (114). However in view of the similar pyrogenic action of PG in several species, which themselves show a variety of temperature responses to the monoamines, it is unlikely that there is a direct association between NA, 5-HT and PG.

There is no evidence yet that PG synthesis and release play any part in normal temperature regulation in the absence of pyrogens. The PG concentration in the CSF of animals at ordinary laboratory temperatures is only 1 ng/ml or less (73) but this may not be the case in hotter or cooler conditions. Relevant measurements do not seem to have been made.

PGE_1 and E_2 have many central effects not concerned with fever. Microinjections of PGE_1 into the PO/AH area also act on the central control systems for food and water intake. Rats were found to eat and drink less after injections of 1 μg (in 1 μl) and there was also a rise of 2°C in their rectal temperature (10).

F. Antipyretics

Although the use of drugs such as salicylate to alleviate fevers has an ancient history, our knowledge of how and where they act was minimal up to about 6 years ago. If a fever is generated as has just been outlined, then an antipyretic drug might act at one or more of the various stages. It could act: 1) To inhibit the production of EnP; 2) to prevent EnP from reaching its target in the PO/AH; 3) to prevent EnP from acting upon cells in the PO/AH; 4) to inhibit the release or synthesis of PG; 5) directly on the central neurons driving thermoregulatory control actions; 6) directly on thermoregulatory effector organs such as the vasoconstricted skin blood vessels, the shivering muscles or their peripheral innervation.

As has already been mentioned, there is evidence for the fourth possibility as being one and probably the main action of antipyretics. The information available about each of the six hypothetical modes of action will now be considered; most authors have used sodium salicylate as the experimental antipyretic, with rabbits and man as experimental species.

1. Production of endogenous pyrogen. Gander et al. (98) incubated bacterial

ExP with rabbit leucocytes *in vitro* and found that salicylate reduced the yield of EnP. Their observation indicated an action on the production of EnP and was supported by experiments on rabbits given pretreatment with salicylate before fevers caused by ExP or EnP: the first type of fever was reduced by the salicylate, but not the second. It was suggested that the salicylate did not act centrally, but only by reducing the formation of EnP. These experiments in rabbits do not seem to have been repeated, but in cats the yield of EnP from incubation *in vitro* was not affected by the presence of acetominophen or salicylate (41). The conclusion reached by Gander *et al.* (98) is not consistent with the results of other experiments in rabbits and man (1, 52) in which steady-state fevers were produced by continuous infusion of EnP. Intravenous injections of sodium salicylate caused prompt and progressive reductions of the raised temperature and the defervescence was dose-dependent. Since there was no production *in vivo* of EnP in these experiments it is clear that the salicylate must have been acting on some other part of the fever-producing process.

2. Entry to hypothalamus. Pretreatment of rabbits with salicylate reduces the fever caused by intravenous EnP, but not by EnP injected into the cerebral ventricles (45). This difference in response suggested that salicylate was interfering with the passage of EnP from the circulation into brain tissue, but later results (50) have not confirmed the suggestion. Salicylate brought temperature down in rabbits in which EnP was given by the ICV route and therefore had direct access to the hypothalamus. Indeed, similar effects were seen whether the salicylate was given intravenously or into the cerebral ventricles. A central site of action for salicylate is therefore indicated.

3. Action on preoptic and anterior hypothalamic neurons. In just the same area, the medial part of the PO/AH, in which microinjections of EnP have their greatest effect, microinjections containing up to 30 μg of sodium salicylate cause an immediate fall in the fever produced by a constant intravenous infusion of EnP (53). Thus at least part, and probably most, of the antipyretic effect of salicylate is locally mediated in this area.

There is neurophysiological data, already quoted (37, 68, 204) which shows that the PO/AH temperature-responsive neurons have their characteristics changed when a pyrogen is given systemically: warm-excited neurons become less sensitive and cold-excited neurons more sensitive to local temperature. In some of the experiments on cats (204) the animals were given aspirin, whereupon the altered characteristics of the neurons were restored to normal. Presumably the restoration is associated with the ability of aspirin to inhibit PG synthesis.

4. Prostaglandins and antipyretics. As already discussed there seems to be a close association between the action of EnP and the release or increased synthesis of PGE_2, but as yet the precise local mechanisms can only be a question for speculation. One of the properties of antipyretics, as shown by Flower and Vane (90) and Vane (193), is to inhibit the PG synthetase enzyme. The inhibition was observed *in vitro* for the three drugs, paracetamol, indomethacin and aspirin, acting on the brain synthetase from dogs and rabbits. The same three drugs were used recently in observations on cats given fever by ICV or intravenous injections of bacterial ExP (73). Samples of cisternal CSF were taken before giving pyrogen, during the ensuing fever and when the fever was brought down by intraperitoneal injection of one of the antipyretics. The PG content in the CSF rose several-fold during the fever but was reduced to the low, pre-fever level when an antipyretic was given. The implication was that in an experimental fever, as well as *in vitro*, antipyretics inhibit the synthesis or release of

PG. It might be expected that the inhibition would take place in the same hypothalamic area as the injection of exogenous PG causes a fever, namely the familiar PO/AH region. This has yet to be demonstrated.

In addition to reducing the synthesis of PG, it is possible that antipyretics could also function by blocking the action of PG on its target neurons. This possibility has been rendered unlikely by Milton and Wendlandt (140) who found that paracetamol (50 mg/kg) failed to reduce the fever caused by ICV injections of PGE_1 or E_2.

An explanation seems to be almost in sight for the mechanisms by which pyrogens generate a fever and antipyretics reduce a fever. A number of outstanding questions now require answers: such answers will probably come only from experiments conducted at the cellular level, *i.e.*, with microelectrodes and the microiontophoretic application of substances onto single hypothalamic neurons. For example, does EnP act only on the temperature responsive neurons as the experiments with systemic ExP suggest? Do these neurons in turn release PG or are other cells synaptically or directly stimulated to do so? On which cell types do the antipyretics act?

5. Central action on effector pathways. Despite this recent and persuasive evidence involving PG, other possible sites and mechanisms of antipyretic action must also be considered. It is quite conceivable that a fever could be reduced by an inhibitory action on the central neurons which excite the control actions (fig. 1) for generating a fever. To test this possibility, the relevant neurons were excited by locally cooling the hypothalamus of conscious rabbits (50). Rectal temperature rose by 1°C and remained raised for as long as the cooling was maintained. When salicylate was infused intravenously it had no effect on the raised level of body temperature. Another type of "fever" which does not involve pyrogens can be produced by perfusing the ventricular system of a cat with a solution containing sodium but no calcium (see section V). Under these circumstances no PG is released and intraperitoneal injections of antipyretics have no effect (62).

6. Peripheral action on effector mechanisms. The experiments with brain cooling just referred to (50) also make it unlikely that salicylate lowers temperature by directly inhibiting shivering and vasoconstriction in the periphery. Both mechanisms were activated by the brain cooling but neither was affected when salicylate was infused. There is also much similar evidence to show that salicylate has no effect on temperature of resting animals or man, irrespective of the route of administration (52, 53, 151, 172). The elevated temperature of an exercising man is also unaffected by salicylate (65).

Nearly all the observations therefore suggest that antipyretic drugs, in particular salicylate, are only effective in the presence of a pyrogen-induced fever. It is likely that their mechanism of action is to inhibit the synthesis of prostaglandin. However, there is one report that acetophenetidin and phenylbutazone can affect the body temperature of afebrile rabbits when ordinary therapeutic doses are given in cold or hot conditions (206). In the cold, treated rabbits showed a larger drop in rectal temperature and in the heat they had a larger rise than controls. These effects could be due to the use of cold and hot conditions; all the work with salicylates cited above was done at ordinary laboratory temperatures. There is another report concerning experiments with salicylate on rats exposed to the cold (177). A clear dose-dependent fall in body temperature was seen in afebrile rats with doses of salicylate ranging from 60 to 300 mg/kg. Whether the action of the salicylate was peripheral or central is uncertain, nor is it yet clear whether the effect is peculiar to rats. Since the possibility still exists that prostaglandins may be involved in

normal thermoregulation as well as in fever, these results could be explained if a prostaglandin were to be released on cold exposure.

G. Conclusions

On the evidence reviewed, it seems as if the generation and alleviation of fever is one of the better understood aspects of temperature regulation or rather its malfunction. However we will need answers to questions such as: Where is EnP produced in a natural fever? How does EnP enter the nervous system? What are the details of the interactions between EnP, PG and neurons?

V. The Central Actions of Cations on Temperature Regulation

Since 1970 there has been a series of papers demonstrating that the temperature of various species can be dramatically raised or lowered by altering the relative concentrations of sodium and calcium ions in the ventricular system. It has been suggested that the ratio of those ions determines the level of the set-point for temperature and that the neurons on which the ions act to achieve this control are to be found in the posterior hypothalamus.

When the ventricular system of cats was perfused with 0.9% NaCl solution containing no calcium there was an immediate and steep rise in body temperature due to vasoconstriction and shivering (82). In similar experiments on rabbits (83) the same hyperthermic effect of a low Ca^{++} perfusate was observed and, in addition, when the Ca^{++} in the fluid was raised from 1.25 mM to 5 mM, there was a fall in temperature. Similar ionic effects on temperature have been found in other species such as monkey (157), rats (148), hamsters (149) and ground squirrels (104), but sheep showed effects only on eating and drinking, not temperature (179). The effective Na^{+} and Ca^{++} concentrations in the ventricles have also been changed in other ways with similar effects on temperature. When the Ca^{++} chelating agents, ethylene diamine tetraacetic acid disodium or ethylene glycol tetraacetic acid, were given by ICV injection into cats or monkeys, body temperature rose (39, 161); when transmembrane sodium flux was diminished by giving tetrodotoxin then cats displayed a fall in temperature (40).

A. Site of action

Contrary to expectations, the site of action of the ions is not in the PO/AH region where most other stimulants to thermoregulation have been shown to act. By making microperfusions with push-pull cannulae, Myers and Veale (155) and Myers *et al.* (157) have shown that only in the posterior hypothalamus at the level of the mammillary bodies and close to the midline was it possible to raise or lower the animal's temperature by perfusion with excess Na^{+} or Ca^{++} respectively. Gross changes in the ionic content of the perfusing fluid were needed to provoke body temperature changes, especially the hypothermia caused by excess Ca^{++}; the concentration of this ion in the Krebs-Ringer solution was increased by factors of up to 20 or even 37. In experiments on monkeys (157) the ratio of the Na^{+} and Ca^{++} concentrations in the perfusate was found to be the critical factor affecting temperature, rather than the actual concentrations of these ions. Body temperature was unaffected by a doubling of both Na^{+} and Ca^{++} concentrations or by the complete absence of both ions during perfusion with isotonic sucrose solution. The posterior site of action of ions is thought to indicate that the ions act on cell bodies or synapses in a descending pathway which is, or forms part of, the output from the more anterior PO/AH area.

The posterior site located in monkeys is not the only ion-sensitive region in other species, because in ground squirrels, Hanegan and Williams (104) found that perfusion of the PO/AH region with high Ca^{++}

solutions also caused body temperature to fall. The fall was greater at cold ambient temperature and was interpreted as being due to a nonspecific depressive action of the raised Ca^{++}.

B. Ionic set-point shifts

Monkeys may have their body temperatures raised or lowered for hours if given repeated microperfusions with excess Na^{+} or Ca^{++} (161). It was reasoned that the sustained high or low levels corresponded to new set-points achieved by the action of the ions. When monkeys in these hyper- or hypothermic states were given cold or hot water by stomach tube, there was a transient shift in brain temperature. The animals then made appropriate shivering or vasodilator responses and brain temperature returned to where it had been before the thermal load was given. Myers and Yaksh (161) claim on this evidence that the monkeys were thermoregulating normally about a new set-point created by the ionic injections.

Such qualitative tests cannot reveal whether the animal was responding normally to internal thermal changes because none of the thermoregulatory responses was actually measured. Much more sophisticated experiments are necessary to establish the significance of hypothalamic ion concentrations in the control of body temperature. For example, quantitative calorimetric measurements should be used to establish the relationship between thermal load and response in animals in which body temperature was displaced by excess Ca^{++} or Na^{+}.

C. Conclusions

The evidence accumulated so far cannot be said to have established that local concentration of Na^{+} and Ca^{++} in a particular part of the posterior hypothalamus determines the set-point for temperature. The data could equally well be interpreted in terms of a nonspecific action of the ions when their concentrations are changed in the vicinity of some neurons which govern the control actions for heat loss and heat conservation. Very gross changes in the ionic concentration are needed to produce changes in body temperature. How and indeed whether such changes can occur in real life has yet to be demonstrated. The only indirect evidence (154) which could be said to support the proposal is that the ionic concentrations in ventricular CSF changed in the predicted fashion when a bacterial pyrogen was given: Ca^{++} was released by some tissue and Na^{+} was retained as the fever developed. Whether these changes were due to neuronal tissue or to a modified production of CSF by the choroid plexus is not clear; the perfusion route was from one lateral ventricle to the other.

Recently it has been found (64) that the temperature effects of excess Ca^{++} and Na^{+} in the ventricular system could be reversed by raising the ambient temperature. In dogs kept at 21°C, high Ca^{++} or Na^{+} caused hypo- or hyperthermia respectively, as already described for other species. However at 28 to 33°C these actions were reversed, which would not be the case if the ions caused a shift in the set-point of the temperature system.

Other bodily functions can be activated or suppressed by raising the Na^{+} or Ca^{++} concentrations in precisely the same posterior hypothalamic area from which temperature effects are obtained (frontal plane 10 in the cat). Increased Ca^{++} leads to sleep, sedation and catatonia (195), a situation in which hypothermia would be expected to develop. Increased Na^{+} causes arousal, rage and a defense reaction, all of which should at the same time cause hyperthermia. Feeding can be induced in satiated cats by the infusion of both high Na^{+} *and* high Ca^{++} (156) and in rats raised intraventricular Ca^{++} causes vigorous eating and drinking (147). Ionic effects on feeding have also been reported in sheep (179) with very small concentration changes in the third ventricle.

The literature on the importance of calcium in transmitter release (173a) and on the excitability of neuronal membranes (31, 94, 95, 121) clearly shows that this ion has opposite actions on these two processes. Raising the extracellular calcium enhances transmitter release but stabilizes membranes so that they become less excitable. Low calcium has opposite effects. In an isolated preparation of the mammalian cerebral cortex the effect of Ca^{++} on transmitter release is known to be powerful and nonlinear (171). In contrast, sodium ions have a relatively weak action on transmitter release from the superior cervical ganglion of the cat (120).

Finally, it must be emphasised that our present understanding of the set-point concept as applied to the control system for body temperature is still far from clear (113). The term set-point is borrowed from control theory to describe some reference level around which body temperature is regulated. There is uncertainty as to whether this reference has a thermal or nonthermal basis and even whether it exists at all (102, 141). To use a telling phrase from Walshe (200), we must beware of "anatomizing an abstraction."

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