EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE TURNOVER OF NORADRENALINE IN HYPOTHALAMUS AND OTHER AREAS OF RAT BRAIN

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SUMMARY

1. The hypothesis that noradrenaline (NA) may be a transmitter in the temperature regulating centre in the hypothalamus is based on the changes in rectal temperature induced by injection of large doses of NA into the brain. As an alternative approach, the effect of environmental temperature on the rate of turnover of endogenous NA in the hypothalamus has been studied.

2. Small amounts of tritium labelled noradrenaline [^{3}H]NA were injected into the c.s.f. of rats in order to label radioactively the endogenous NA in the brain. The rats were then exposed to environmental temperatures of 9, 17, 24 and 32° C. The rates of disappearance of [^{3}H]NA from discrete areas of brain were taken as indices of the rates of turnover of endogenous NA in those areas.

3. The rate of disappearance of [^{3}H]NA from the hypothalamus was three times as fast at 9 and 32° C as at 17 or 24° C. There were no such significant differences from the pre-optic area or 'rest of brain' (whole brain minus hypothalamus, pre-optic area, cerebellum and medulla).

4. The endogenous concentrations of NA were not altered by the experimental procedures in any of the areas of brain studied.

5. The rats maintained normal rectal temperatures at environmental temperatures of 9, 17 and 24° C but became 2·8° C hyperthermic at 32° C.

6. It is concluded that mild conditions of both heat and cold resulted in an increased turnover of NA in specific nerve terminals in the hypothalamus. Since the rats were thermoregulating normally, the nerve terminals involved are regarded as forming a part of the central temperature regulating centre.

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INTRODUCTION

The suggestion by Feldberg & Myers (1963) that NA and 5-hydroxy-tryptamine (5-HT) may be involved in the central regulation of body temperature has aroused considerable interest. In the rat, injection of NA into the cerebral ventricles causes a fall in body temperature followed by a rise above the initial level, while 5-HT causes only a fall in temperature (Feldberg & Lotti, 1967). The pattern of response varies according to species (Cooper, Cranston & Honour, 1965; Feldberg, Hellon & Lotti, 1967; Findlay & Thompson, 1968), but in the cat the two amines have been found to be most effective when injected directly into the anterior hypothalamus (Feldberg & Myers, 1965). This is compatible with a large volume of evidence implicating the hypothalamus as a temperature regulating centre in the brain (see Bligh, 1966). In addition, the hypothalamus is known to contain a relatively high concentration of NA (Vogt, 1954; Laverty & Sharman, 1965), much of it within nerve terminals (Fuxe, 1965).

Attempts to demonstrate changes in the concentration of NA in the whole brain or hypothalamus of the rat in response to both cold and heat have met with variable success (Maynert & Levi, 1964; Beauvallet, Legrand & Fugazza, 1967; Ingenito & Bonnycastle, 1967). It has been shown, however, that the concentration of NA in the brain can remain constant while the rates of release and synthesis of NA are increased (Gordon, Spector, Sjoerdsma & Udenfriend, 1966). Estimates of the rate of turnover of NA would, therefore, provide more sensitive indices of neuronal activity. Increases in the turnover of NA in whole brains of rats exposed to either cold (Costa & Neff, 1966; Gordon *et al.* 1966; Duce, Crabai, Vargiu, Piras, Adamo & Gessa, 1968; Reid, 1968) or heat (Corrodi, Fuxe & Hökfelt, 1967) have been reported, but no measurements were made on the hypothalamus alone. It is likely, however, that thermoregulation was impaired in these experiments (Corrodi *et al.* 1967) since the method used to measure turnover involved inhibition of the synthesis of NA, peripherally as well as centrally, with drugs such as α-methyltyrosine.

In the experiments to be described, a different method was used. Small amounts of [^{3}H]NA were injected into the c.s.f. of the rat in order to radio-actively label the NA stores in the brain. The rates of disappearance of [^{3}H]NA from discrete areas of the brain were then used as indices of the rate of turnover of NA in those areas. The results of an initial series of experiments, using this technique, have already been published (Simmonds & Iversen, 1969).

METHODS

Male Wistar rats (200–250 g) were kept in the laboratory at an environmental temperature between 20 and 24° C for about 18 hr before the start of an experiment.

Turnover of [³H]NA. Rats were anaesthetized with ether and injected intracisternally (Schanberg, Schildkraut & Kopin, 1967) with small amounts (2·5 μc, 0·044 μg) of (±)-7-[³H]noradrenaline (NEN Chemicals, GmbH, Dreieichenhain, W. Germany) in 20 μl. artificial c.s.f. (Merlis, 1940) containing 0·001% ethylenediaminetetraacetate and 0·002% ascorbic acid at pH 6·0. After 30 min at room temperature, when the rats had recovered from the anaesthetic, they were placed in individual cages in an environment of 9, 17, 24 or 32° C controlled to within ± 2° C. During this period no food or water was provided. At 2, 4 and 6 hr after injection, the rats were stunned and decapitated immediately after having their rectal temperatures measured with a Grant Thermistor probe inserted 5 cm into the rectum. The brains were rapidly removed, rinsed in ice-cold sodium chloride solution (0·9 g/100 ml.),

TABLE 1. Mean weights ± s.d. of the dissected areas of brain

	Tissue wt. (mg)
Pre-optic area	35 ± 5
Anterior hypothalamus	37 ± 5
Posterior hypothalamus	34 ± 5
Whole hypothalamus	67 ± 8
'Rest of brain'	1204 ± 56

blotted and chilled. Cerebellum and medulla were discarded since they contained a disproportionately high concentration of [³H]NA, because of their close proximity to the site of injection. A single block of tissue comprising most of the pre-optic area and hypothalamus was dissected from the rest of the brain as described for the hypothalamus by Glowinski & Iversen (1966) but with the rostral limit extended about 1·5 mm further rostrally to include more of the pre-optic area (de Groot, 1959). Pre-optic area was separated from hypothalamus by transverse section through the caudal end of the optic chiasma. In those experiments where the hypothalamus was divided into anterior and posterior parts, a transverse section was made across the base of the infundibulum. The remaining areas of the brain, i.e. whole brain minus pre-optic area, hypothalamus, cerebellum and medulla, are described as 'rest of brain'. The weights of the dissected areas are given in Table 1.

Extraction and isolation of [³H]NA. The tissue samples were rapidly homogenized in 0·4 N perchloric acid containing 0·1% ethylenediaminetetraacetic acid. Pre-optic area, anterior hypothalamus and posterior hypothalamus were extracted in 0·5 ml., whole hypothalamus in 1·0 ml. and 'rest of brain' in 12·0 ml. acid. The contribution of tissue water to the final volumes of the extracts was taken into account in the subsequent calculations. After centrifugation and removal of 0·1 ml. aliquots of the supernatant for estimation of total tritium, the remainder of the pre-optic and hypothalamic supernatants was reserved and each pellet was resuspended in a volume of 0·4 N perchloric acid equal to that used for the first extraction. After further centrifugation, the second supernatant was added to the first. Ascorbic acid was added to these extracts and to 5 ml. aliquots of the 'rest of brain' supernatants to a concentration of 0·01%. Neutralization to pH 5·5–6·0 was made with KOH and K_2CO_3 solutions so that potassium perchlorate was precipitated. This was done using either a pH meter or an internal indicator (1 part bromocresol green + 3 parts chlorophenol red). The neutralized solutions were kept at 4° C to allow complete precipitation of potassium perchlorate and the supernatants were then poured onto columns of Zeo-Karb 225 cation exchange resin, 200–400 mesh, in the Na⁺ form. The resin was pretreated in bulk according to the procedure of Häggendal (1962). The resin columns for the 'rest of brain' extracts

were 5 cm long × 6 mm diameter and, following passage of the neutralized extracts, were washed successively with 15 ml. water and 7·5 ml. 1 N-HCl. The NA was then eluted in the next 15 ml. 1 N-HCl. For the smaller volumes of the pre-optic and hypothalamic extracts, columns of resin 5 cm long × 2·5 mm diameter were used (Fig. 1). Following passage of the neutralized extracts, the resin was washed with 3 ml. water followed by 2 ml. 1 N-HCl. The NA was then eluted in the next 3 ml. 1 N-HCl. The entire acid eluates from the smaller columns and 2 ml. aliquots of those from the larger columns were evaporated to dryness *in vacuo* over flake sodium hydroxide and the dried material was redissolved in 0·2 ml. water.
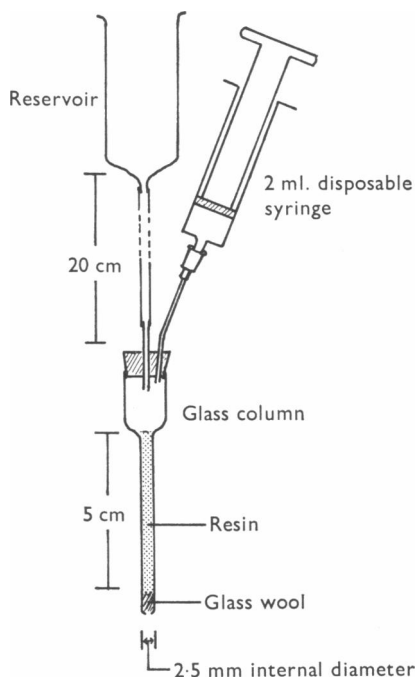

Fig. 1. Apparatus for the isolation of small amounts of NA from tissue extracts on Zeo-Karb 225 cation exchange resin. The neutralized extract is poured into the reservoir. Air is then withdrawn, by means of the syringe, from the space above the resin so that the extract begins to drip onto the resin. The friction between plunger and barrel of disposable syringes is sufficient to prevent any further movement of the plunger. In this way, a pressure head of the extract is set up. When only a few drops of the extract remain to pass through the resin, the next addition of liquid may be made to the reservoir. The column of air between the two liquids prevents mixing and the new pressure head speeds the passage of the last few drops of extract through the resin. When the meniscus above the resin has disappeared, the liquid in the reservoir is allowed to drip onto the resin by further withdrawing the plunger in the syringe. Care must be taken, when using resins of large particle size, not to allow the surface of the resin to remain dry for too long with a pressure head above it, otherwise air may be forced between the particles and thereby isolate some of the resin from contact with subsequent additions of liquid.

Using 200–400 mesh Zeo-Karb 225 (Na$^+$ form), decanted repeatedly to yield a particle size which sediments in less than $3\frac{1}{2}$ min, 2 ml. liquid takes about 1 hr to pass through the resin.

To these samples, and to the aliquots taken for estimation of total tritium, were added 4 ml. ethoxyethanol and 10 ml. of a 0·4 % (w/v) solution of butyl-PBD (CIBA) (2-(4′-*t*-butyl-phenyl)-5-(4″-biphenylyl)-1,3,4-oxdiazole) in toluene. The tritium content of the samples was estimated in a Nuclear Chicago liquid scintillation counter. The recovery of NA from the resin was estimated by adding a known amount of [³H]NA to brain extracts from untreated rats. The percentage of added tritium appearing in the acid eluate was then determined. The recovery varied a little between batches of resin, but was usually between 60 and 75 %. This was taken into account in the calculation of [³H]NA content of the tissues, expressed as $\mu\mu c$/mg tissue.

Estimation of endogenous NA. A separate series of experiments was performed to deter-mine the effects of environmental temperature and the injection procedure on the concen-tration of endogenous NA in the brain. Accordingly, the rats were treated exactly as before except that unlabelled NA, instead of [³H]NA, was injected and all animals were killed 6 hr after injection. Brains were dissected and homogenized as before except that the pre-optic areas from two rats were combined and the 'rest of brain' samples were extracted in half the previous volumes. The NA in extracts of all three brain regions was purified by passage through small columns as described above. Aliquots of 1 ml. 1 N-HCl eluates were assayed for NA by the method of Euler & Lishajko (1961).

Statistical analysis. The concentrations of [³H]NA in the tissues were expressed as a per-centage of the mean concentration at 2 hr after injection. These values were converted to logarithms and the regression on time calculated by the method of least squares. The slope of the regression and its standard error were obtained (Goldstein, 1964) to enable further calculation of the rate constant (k) of decline of [³H]NA concentration from the equation: $k = 2·303 \times$ slope (Brodie, Costa, Dlabac, Neff & Smookler, 1966). The significance of the differences between values of k was determined by Student's t test.

RESULTS

Rectal temperatures. The rectal temperatures of untreated control rats and of experimental rats at various times after injection are shown in Table 2. Only those rats exposed to 32° C showed a significant change ($P < 0·05$) in rectal temperature, compared with controls. The hyper-thermia observed at 32° C was established within the first 1½ hr of ex-posure to the temperature and remained approximately constant for at least a further 4 hr.

Turnover of [³H]NA. The fall, with time, in the concentration of [³H]NA in the hypothalamus at different environmental temperatures is shown in Fig. 2. Since, at each temperature, the points fall approximately on a straight line, the decline has been treated as a single exponential. Similar straight line plots were obtained for the pre-optic area and the 'rest of brain' samples. Calculated values of the rate constant (k) of decline in con-centration of [³H]NA for each of the tissues are shown in Fig. 3. Values for the half-time ($t_{\frac{1}{2}}$) of disappearance of [³H]NA are given in Table 3.

There were no significant differences ($P > 0·1$) between the values of k at the different environmental temperatures in either the pre-optic area or 'rest of brain'. In the whole hypothalamus, however, k was significantly ($P < 0·005$) higher at both 32 and 9° C than at 17 or 24° C. These increases

TABLE 2. Effect of environmental temperature on the rectal temperatures of rats 2, 4 and 6 hr after injection of [³H]NA. The duration of exposure to the temperature was 1·5, 3·5 and 5·5 hr respectively. The values given are means ± S.D.

	Rectal temperature			
	Time after injection			
Environmental temperature (° C)	2 hr (°C)	4 hr (° C)	6 hr (° C)	Average (° C)
9	37·7 ± 1·1	38·1 ± 1·0	37·8 ± 0·9	37·8 ± 0·9
17	37·6 ± 0·4	36·9 ± 1·0	37·7 ± 1·2	37·4 ± 0·9
24	37·7 ± 0·5	—	37·9 ± 0·6	37·8 ± 0·6
32	40·4 ± 0·6	40·3 ± 0·8	41·0 ± 0·7	40·6 ± 0·8*
Untreated control at room temperature (20–24° C)				37·7 ± 0·4

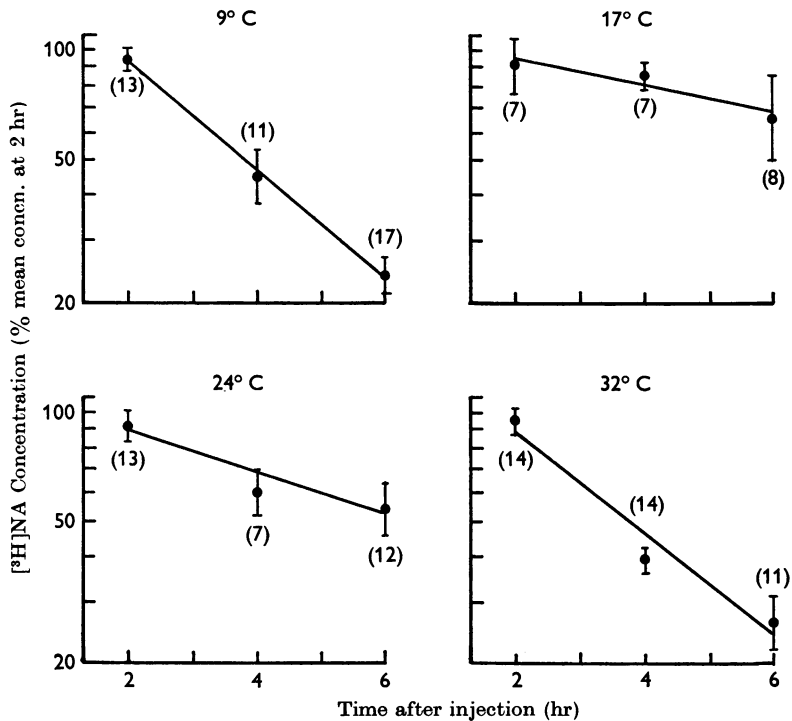* Significantly different from untreated control ($P < 0.05$).

Fig. 2. Effect of environmental temperature on the fall, with time, in the concentration of [³H]NA in the hypothalamus of the rat following intracisternal injection of [³H]NA. Rats were exposed to 9, 17, 24 and 32° C from 30 min after the time of injection. The concentration of [³H]NA is expressed as a percentage of the mean concentration at 2 hr and each point is the mean ± S.E. of the logarithms of the values. The numbers of values contributing to each point are shown in brackets. The lines were calculated from the logarithms of the values by the method of least squares.

occurred in both anterior and posterior parts of the hypothalamus, and although the rates of disappearance of [³H]NA were generally slightly higher in the posterior hypothalamus, the percentage increases at 9 or 32° C were similar in both anterior and posterior parts.

Endogenous NA. The concentrations of endogenous NA in the pre-optic, hypothalamic and 'rest of brain' areas are shown in Fig. 4. In none of these areas were there any significant differences ($P > 0 \cdot 1$) between the NA
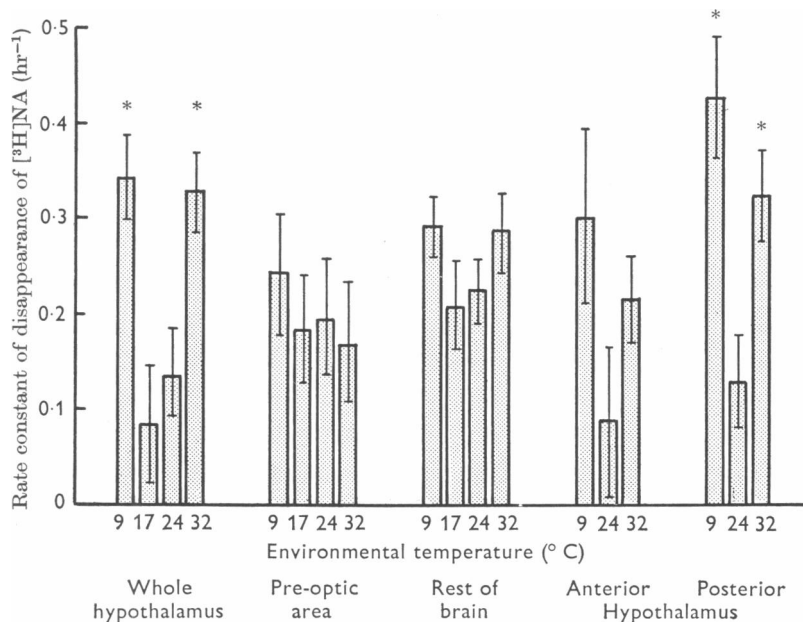

Fig. 3. Effect of environmental temperature on the rate constant (k) of decline in [³H]NA concentration in whole hypothalamus, pre-optic area, 'rest of brain' (i.e. whole brain minus hypothalamus, pre-optic area, cerebellum and medulla) and anterior and posterior hypothalamus. Values of rate constant $\pm$ S.E. were calculated from twenty-two to forty-one estimates of [³H]NA concentration obtained 2, 4 and 6 hr after intracisternal injection of [³H]NA.

* $P < 0 \cdot 01$ when compared with value at 24° C.

TABLE 3. Half-times ($t_{\frac{1}{2}}$) of disappearance of [³H]NA from various areas of rat brain between 2 and 6 hr after intracisternal injection of [³H]NA. Half-time was calculated from the results presented in Fig. 3 according to the equation: $t_{\frac{1}{2}} = 0 \cdot 693/k$

Environmental temperature	$t_{\frac{1}{2}}$			
	9° C (hr)	17° C (hr)	24° C (hr)	32° C (hr)
Pre-optic area	2·85	3·80	3·49	4·07
Whole hypothalamus	2·01	8·31	5·06	2·12
Anterior hypothalamus	2·30	—	8·04	3·16
Posterior hypothalamus	1·63	—	5·31	2·14
'Rest of brain'	2·38	3·32	3·04	2·41

concentrations in control rats and those in experimental rats exposed to 9, 24 or 32° C for 5½ hr.
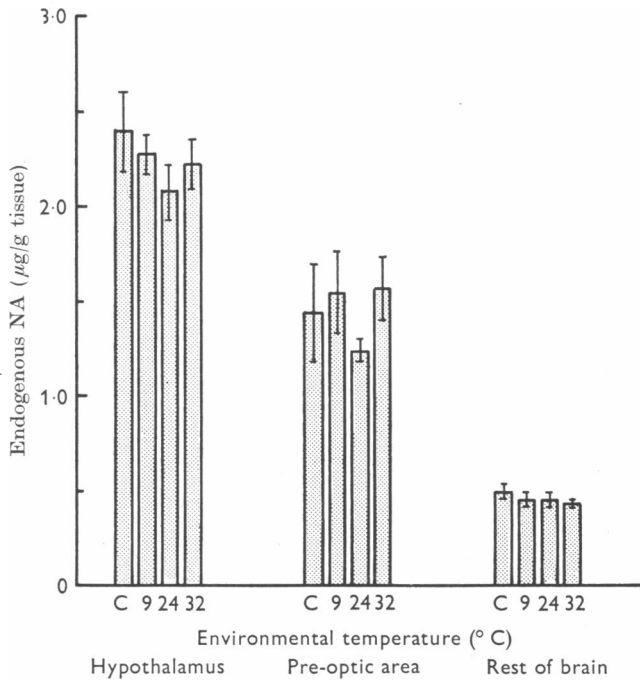

Fig. 4. The concentrations of endogenous NA in hypothalamus, pre-optic area and 'rest of brain' (i.e. whole brain minus hypothalamus, pre-optic area, cere-bellum and medulla) of untreated control rats (C) and rats exposed to 9, 24 or 32° C for 5½ hr following intracisternal injection of 0·044 μg NA under ether anaesthesia. Values given are mean ±s.e. of eight to eleven values for hypotha-lamus and 'rest of brain' and of four to five values for pre-optic area.

DISCUSSION

Normal rats exposed to moderate cold (3 or 4° C) or heat (34 and 40° C) show no change in the concentration of NA in the brain (Maynert & Levi, 1964; Gordon *et al.* 1966; Ingenito & Bonnycastle, 1967), although extreme conditions of cold exposure can cause a fall (Maynert & Levi, 1964). The estimates of endogenous NA obtained in the present experiments, similarly, showed no change with environmental temperature and the values obtained are generally in agreement with those of other authors (Glowinski & Iversen, 1966; Kovacsics & Saelens, 1968; Maickel, Cox, Saillant & Miller, 1968). The maintenance of stable concentrations of NA in different areas of the brain implies that the utilization of NA is exactly balanced by new synthesis. Thus, turnover may vary without causing any measur-able change in the concentration of endogenous NA. A number of different

techniques have been used to measure the turnover of NA in the brain (see Iversen & Simmonds, 1969). The technique used in the present experiments, based on that of Iversen & Glowinski (1966), has the advantage over methods involving inhibition of the synthesis of NA that thermoregulation remained unimpaired. The amount of [³H]NA injected was much smaller than that required to induce a change in the body temperature of the rat (Feldberg & Lotti, 1967), but was sufficient to label the NA stores throughout the brain. Previous studies have established that [³H]NA is selectively accumulated by NA-containing neurones in the rat brain (Glowinski & Iversen, 1966; Aghajanian & Bloom, 1967; Reivich & Glowinski, 1967). There are, however, certain limitations to this method. The NA-containing neurones are not all equally well labelled (Fuxe & Ungerstedt, 1966; Iversen & Simmonds, 1969) so that the turnover results are weighted by the strongly labelled neurones. The method also assumes that the NA in each neurone is in a single homogeneous pool, an assumption which has been questioned recently (Sedvall, Weise & Kopin, 1968; Ngai, Neff & Costa, 1968). The absolute rate of turnover of endogenous NA in the various areas of brain has, therefore, not been calculated in the present experiments but the rate constant (k) of decline in [³H]NA concentration has been regarded as an index of NA turnover, reflecting the activity of the neurones involved.

In order to calculate values of k, the disappearance of [³H]NA must be treated as a single exponential. Since, in the brain, there is a large number of NA-containing neurones subserving different functions, with various degrees of activity, the decline in concentration of [³H]NA is multi-exponential. In each of the areas of brain examined, however, the mean concentrations of [³H]NA at 2, 4 and 6 hr after injection were close to a single exponential line. As a result, it was considered valid to use k as an expression of the over-all rate of disappearance of [³H]NA from each area.

When significant differences between the values of k for an area are obtained, these must reflect significant changes in the rate of turnover of NA in a substantial proportion of the neurones labelled with [³H]NA in that area. Significant increases in the rate of turnover of NA were found only in the hypothalamus at environmental temperatures of 9 and 32° C when compared with the rate of turnover at 24° C. No such responses were seen in the adjacent pre-optic area or in the 'rest of brain' (whole brain minus hypothalamus, pre-optic area, cerebellum and medulla). Thus, it appears that NA is released from neurones in the hypothalamus in increased amounts under conditions of both heat and cold. In an initial series of experiments (Simmonds & Iversen, 1969), however, the turnover of NA did not rise in the cold and the rats became hypothermic. No explanation can be found for the apparent failure of thermoregulation in the cold in the

earlier study but the results obtained in the present study represent the more usual response to cold.

Although stress is also known to increase the turnover of NA in the brain, the stress of electric shock treatment does so throughout the rat brain (Thierry, Javoy, Glowinski & Kety, 1968). Since, in the present experiments, the increases in turnover of brain NA occurred selectively in the hypothalamus it is probable that these responses were elicited via specific temperature-sensitive pathways rather than by general heat or cold stress. The stress factor cannot be excluded, however, from earlier studies in which the environmental conditions were more severe and where NA turnover was estimated in whole brain (Costa & Neff, 1966; Gordon et al. 1966; Corrodi et al. 1967; Duce et al. 1968; Reid, 1968).

The NA-containing neurones which responded to heat in the present experiments had a similar distribution between anterior and posterior parts of the hypothalamus to those which responded to cold. No such temperature-responsive neurones were detected in the pre-optic area. NA is, therefore, probably not involved in that part of the temperature regulating mechanism which is present in the pre-optic area (Han & Brobeck, 1961). In the hypothalamus, certain cells are sensitive to small changes in local temperature (Satinoff, 1964; Hellon, 1967; Cabanac, Stolwijk & Hardy, 1968), but they probably do not contain NA since the cell bodies of most NA-containing neurones are situated in the lower brain stem (Dahlström & Fuxe, 1964). Axons from some of the NA-containing cell bodies are thought to project to various forebrain structures, including the hypothalamus (Fuxe, 1965; Anden, Dahlström, Fuxe, Larsson, Olsen & Ungerstedt, 1966). Thus, most of the NA in the hypothalamus appears to be located within the nerve terminals. The rate of turnover of NA in the hypothalamus, therefore, is probably regulated, at least in part, from the lower brain stem. The nervous pathways which make synaptic connexions with the NA-containing cell bodies in the lower brain stem have not been clearly defined. Since the ascending pathways from the peripheral temperature receptors are also ill-defined, there is no indication of whether the temperature receptors which modulate the turnover of NA in the hypothalamus are situated centrally or peripherally.

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