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THE EFFECTS OF ETHER STRESS AND BETAMETHASONE TREATMENT ON THE CONCENTRATIONS OF NORADRENALINE AND DOPAMINE IN VARIOUS REGIONS OF THE RAT BRAIN

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1 The effects of ether stress on noradrenaline (NA) and dopamine levels in different regions of the rat brain were studied.

2 Exposure to ether vapour (90 s) caused a significant decrease in the concentration of hypothalamic NA but had no effect on catecholamine (CA) concentrations in the other regions studied.

3 Treatment with betamethasone alone (20 μg/ml) given in the drinking water for 24 h, had no effect on CA levels in the cerebral cortex, midbrain or hypothalamus. However, pretreatment with this dose of steroid prevented the decreases in hypothalamic NA which were normally seen after ether stress and also induced significant increases in the concentration of midbrain NA.

4 The data provide further support for the involvement of NA in the regulation of stress-induced corticotrophin (ACTH) release and indicate that centres other than the hypothalamus may be involved in mediating the inhibitory action of betamethasone on the response to ether stress.

Introduction

Central monoamines have been implicated in the regulation of corticotrophin (ACTH) release, and are found in fairly high concentrations in those regions of the brain that are believed to play a role in this process (e.g. the hypothalamus and midbrain).

Various forms of stressful stimuli have been shown to affect brain catecholamine (CA) levels, although the literature contains some conflicting views. In general, noradrenaline (NA) concentrations have been found to decrease (Ganong & Lorenzen, 1967; Bliss, Ailion & Zwanziger, 1968; Carr & Moore, 1968; Stoner & Elson, 1971), whereas concentrations of dopamine have been reported to be either unchanged (Carr & Moore, 1968; Corrodi, Fuxe, Lidbrink & Olson, 1971) or decreased (Gordon, Spector, Sjoerdsma & Udenfriend, 1966) after stress.

However, most studies of the effects of stress on brain CA concentrations have employed techniques that involve modification of endogenous brain CA levels by the administration of chemical substances, followed by the assessment of pituitary-corticotrophic function before and after stress (Van Loon, Scapagnini, Moberg & Ganong, 1971; Scapagnini, Van Loon, Moberg, Preziosi & Ganong, 1972) or have looked only at CA concentrations in whole brain or limited regions (Barchas & Freedman, 1963). The present study involved the direct estimation of NA and dopamine in seven different regions of the rat brain. It was carried out in order to investigate the effects of a known stressful stimulus (exposure to ether vapour) and the effects of a known inhibitor of the ACTH-releasing activity of this stress (betamethasone), on the concentrations of these neurotransmitter substances.

Methods

Animals

Adult male albino Charles River rats, weighing 200–250 g were used throughout. The animals were housed 2 per cage, at least 5 days before the start of an experiment, and were handled 3 times per week from then onwards (Hodges & Mitchley, 1970b). Food and water were available *ad libitum*.

Stress

The animals were stressed at 09 h 15 min, by placing them in a closed box into which ether vapour was passed. Each animal was exposed to the vapour for

Table 1 Reproducibility of dissection procedure

Brain region	*Mean wt. (mg ± s.e. mean)*
Cerebral cortex	212 ± 6
Cerebellum	282 ± 5
Pons and medulla	218 ± 6
Hypothalamus	51 ± 2
Midbrain	131 ± 4
Corpus striatum	77 ± 2
Hippocampus	96 ± 2

Mean weights for various brain regions dissected from 36 control animals.

90 s, this being sufficient to induce anaesthesia. At specific times thereafter the animals were killed by decapitation. The brains were rapidly removed, chilled in methanol which had been pre-cooled to −60°C by an acetone/solid CO_2 mixture, and kept on solid CO_2 until all the animals in a given experimental group had been killed. A group of 6 untreated control animals was also killed at each time.

Brain dissection and catecholamine assay

The following brain regions were dissected out, according to the scheme outlined by Glowinski & Iversen (1966): cerebral cortex, cerebellum, pons and medulla, midbrain, hypothalamus, corpus striatum and hippocampus. The tissue samples were weighed accurately on a torsion balance and homogenized in 2.5 ml of ice-cold 0.4 N perchloric acid. The reproducibility of the dissection procedure is shown in Table 1. NA and dopamine concentrations were determined fluorimetrically according to the detailed method described by Shellenberger & Gordon (1971).

Betamethasone

Betamethasone in a soluble form (betamethasone sodium phosphate, Glaxo Laboratories Ltd) was administered to rats in the drinking water at a concentration of 20 μg/ml, for a period of 24 hours. Preliminary experiments showed that the total volume of drug solution ingested over the 24 h period remained constant and was equivalent to 450–500 μg of drug/100 g body weight (Vellucci, unpublished observations). The administration of betamethasone in the drinking water provides a non-stressful method of administering the drug and obviates the need for injections. This method was first described by Purves & Sirett (1965) for dexamethasone, and has since been used by other workers in this field (Hodges & Mitchley, 1970a, c; Buckingham & Hodges, 1976).

Results

The effects of ether stress on the concentrations of noradrenaline and dopamine

Of the seven brain regions studied, it was found that significant changes in concentration occurred only in the hypothalamus (Table 2). In this region the concentration of NA fell significantly ($P < 0.01$) from a control value of 6.1 nmol/g, to reach a value of 3.8 nmol/g 10 min after exposure to the stress. The NA concentration then rose to 5.4 and 5.3 nmol/g after 20 and 40 min respectively, but remained significantly ($P < 0.05$) below the control level. It then fell again to reach a value of 3.6 nmol/g at 160 min, which was significantly ($P < 0.001$) lower than that of the corresponding unstressed controls.

Dopamine concentrations were also estimated in the same samples. In all cases the dopamine concentrations were found to remain at a level which did

Table 2 The effect of ether stress on the concentration of noradrenaline (nmol/g tissue wet weight) in different regions of the rat brain

	Time (min) after exposure to ether						
	0	*10*	*20*	*40*	*80*	*160*	*320*
Brain region	(30)	(12)	(6)	(12)	(12)	(12)	(12)
Cerebral cortex	1.2 ± 0.07	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
Cerebellum	0.8 ± 0.08	0.7 ± 0.1	1.0 ± 0.1	0.9 ± 0.06	0.8 ± 0.04	0.8 ± 0.07	0.9 ± 0.1
Pons + medulla	1.5 ± 0.07	1.5 ± 0.1	1.6 ± 0.3	1.3 ± 0.1	1.7 ± 0.2	1.2 ± 0.1	1.4 ± 0.1
Hypothalamus	6.1 ± 0.1	***3.8 ± 0.4	*5.4 ± 0.2	*5.3 ± 0.3	**4.9 ± 0.1	***3.6 ± 0.1	***4.7 ± 0.1
Midbrain	2.3 ± 0.2	1.9 ± 0.3	2.5 ± 0.3	2.4 ± 0.3	2.1 ± 0.1	2.1 ± 0.2	2.0 ± 0.3
Corpus striatum	1.2 ± 0.2	1.3 ± 0.3	1.3 ± 0.1	1.4 ± 0.05	1.5 ± 0.2	1.3 ± 0.1	1.4 ± 0.2
Hippocampus	1.8 ± 0.2	1.7 ± 0.2	1.5 ± 0.3	1.7 ± 0.2	1.8 ± 0.2	1.5 ± 0.2	1.8 ± 0.2

Numbers of animals indicated in parentheses. Values are means ± s.e. mean.
Significantly different from controls, *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3 Dopamine concentrations (nmol/g tissue) in different regions of the rat brain

Brain region	*Dopamine*
Cortex	0.8 ± 0.1
Cerebellum	—*
Pons + medulla	2.3 ± 0.3
Hypothalamus	2.4 ± 0.3
Midbrain	0.4 ± 0.05
Corpus striatum	22.0 ± 3.6
Hippocampus	—*

Each value is the mean (± s.e. mean) of 6 control groups, each containing 6 animals.

* For the cerebellum and hippocampus the fluorimeter readings were not significantly different from the blank value.

not differ significantly from that of the corresponding control groups (Table 3).

The hypothalamic weights of the stressed animals were not significantly different from those of the corresponding controls.

The effect of betamethasone treatment on the response to ether stress

Buckingham & Hodges (1976) have shown, by the use of direct estimates of circulating ACTH, that pretreatment with betamethasone inhibits the stress-induced release of this trophic hormone. Thus, it was of interest to investigate the effects of pretreatment with a suitable dose of betamethasone on the CA response to ether stress. Three brain areas were selected for this study, viz. the cerebral cortex, the midbrain and the hypothalamus, although this does not imply that the other regions are unimportant.

In the cerebral cortex no change occurred in the concentration of NA after betamethasone alone or after betamethasone and ether stress (Figure 1a).

In the midbrain (Figure 1b) neither treatment with betamethasone alone, nor ether stress alone had any effect on the concentration of NA. However, in the groups pretreated with the steroid and then exposed to ether stress there was a significant increase in the concentration of NA, from a control value of 2.3 nmol/g to values of 2.9 nmol/g ($P < 0.01$) and 3.2 nmol/g ($P < 0.001$) after 10 and 160 min, respectively.

Betamethasone alone had no significant effect on hypothalamic NA (Figure 1c). However, the fall in hypothalamic NA which was normally seen 10 and 160 min after exposure to ether vapour was completely inhibited by pretreatment with the steroid. Furthermore, there was a slight, but significant ($P < 0.05$) increase in hypothalamic NA in the steroid-treated group 10 min after stress.

Dopamine levels were also estimated in the same samples. No changes in the concentration of the amine were observed and all values remained at levels which were not significantly different from those of the corresponding controls.

The hypothalamic weights did not differ significantly between groups.

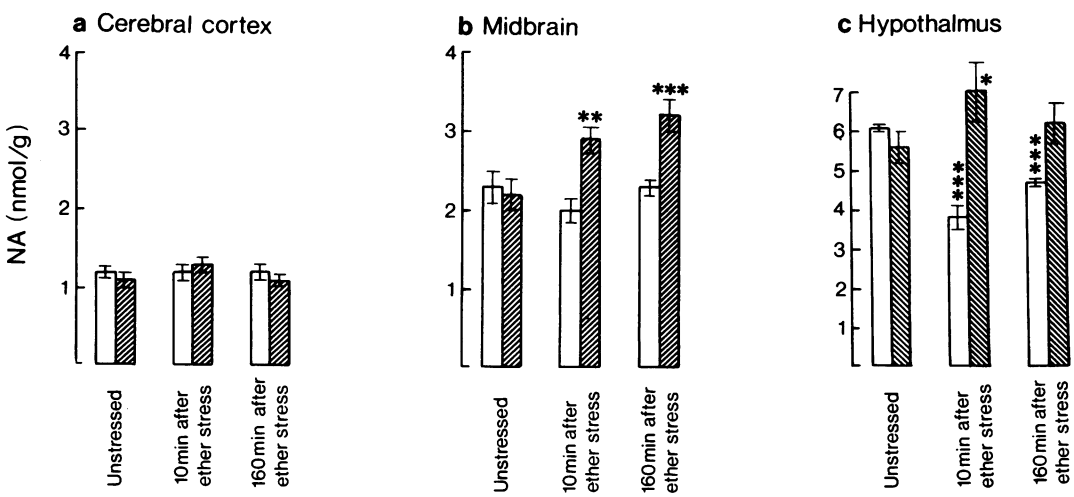

Figure 1 Brain noradrenaline concentrations in untreated rats (open columns) and betamethasone-treated rats (hatched columns) before and after ether stress in (a) cerebral cortex, (b) midbrain and (c) hypothalamus. Each column is the mean of at least 8 determinations. Vertical bars indicate s.e. mean.
Significantly different from corresponding controls. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Discussion

These results show that a significant decrease in the concentration of hypothalamic NA occurred after ether stress, whereas NA levels in the other brain regions, and dopamine levels in all regions, remained unchanged. In addition they show that pretreatment with betamethasone was able to prevent this stress-induced decrease in hypothalamic NA. The results are in agreement with the findings of several authors who have found a decrease in hypothalamic NA after a variety of other stressful stimuli, e.g. emotional stress, foot-shock, forced swimming, restraint, and injection of histamine (Bliss & Zwanziger, 1966; Bliss *et al.*, 1968; Stoner & Elson, 1971), and with those of authors who have found no change in the concentration of dopamine after stress (Bliss *et al.*, 1968; Carr & Moore, 1968; Corrodi *et al.*, 1971). They are also in agreement with the observation that pretreatment of rats with hydrocortisone (cortisol) is capable of preventing the fall in brain NA content which is normally induced by the stress of an electric shock applied to the feet (Shalyapina, 1967).

The results described here support the theory that NA is involved in the regulation of stress-induced ACTH release. However, they do not indicate precisely how NA is involved in the regulation of stress-induced ACTH release. Further studies are therefore necessary in order to determine the relative contribution made by changes in synthesis and/or release of NA and to correlate these changes with alterations in hypothalamo-pituitary adrenal activity. In the light of the recent findings of Palkovits, Kobayashi, Kizer, Jacobowitz & Kopin (1975) who demonstrated that the CA content of the arcuate nucleus was selectively depleted after exposure to three different types of acute stress (i.e. formalin, immobilization and exposure to cold), it would also be of interest to determine whether the decrease in hypothalamic NA observed after ether stress is localized in this hypothalamic nucleus. In the present work only hypothalamic NA exhibited a significant decrease after ether stress, however, it has been reported that a more severe stress, such as foot-shock, can induce decreases in NA throughout the entire brain (Bliss *et al.*, 1968). In addition it cannot be concluded from the present results that CA function in the other regions of the brain remained unaltered, as it is possible for CA turnover rates to change quite markedly without concomitant changes in their absolute levels (Bliss *et al.*, 1968).

It is well established that CA concentrations in different regions of the brain exhibit some degree of diurnal variation (Manshardt & Wurtman, 1968; Simon & George, 1975). However, the observed decreases in hypothalamic NA were thought not to be due to this. This is because, firstly, concentrations of hypothalamic NA have been shown to remain relatively stable during the period of time when the experiments were carried out (Simon & George, 1975), and secondly, because control measurements were made each time and were found not to differ significantly from each other. It is also possible that the observed decreases in hypothalamic NA could have been due to an increase in the blood-content of the hypothalamus. However, no significant differences between the hypothalamic weights of experimental and control animals were found.

Another possible explanation for the finding that NA changes were localized to the hypothalamus, is that ether stress is thought to stimulate ACTH release by acting directly on this region, without the mediation of other parts of the central nervous system (CNS) (Matsuda, Kendall, Duyck & Greer, 1963; Matsuda, Duyck, Kendall & Greer, 1964; Kendall, Allen & Greer, 1965). This seems unlikely, particularly as other investigators have provided evidence which indicates that the response to ether stress is not mediated solely by the hypothalamus, but involves regions such as the midbrain (Royce & Sayers, 1958; Mangili, Motta, Muciacca & Martini, 1965). Mangili *et al.*(1965) found that the corticotrophic response to a stress such as ether was blocked by midbrain transection or by treatment with dexamethasone. Furthermore, Kendall *et al.* (1965) found that, although an increase in plasma corticosterone concentration still occurred in midbrain transected rats subjected to ether stress, it was significantly less than that seen in intact controls, thus indicating that other regions of the CNS are probably necessary for a normal response to this stress. The results reported here demonstrate that whereas neither ether stress, nor betamethasone alone had any effect on midbrain NA levels, betamethasone treatment followed by ether stress induced a significant increase in the NA concentration of this region. Although these results do not show conclusively that betamethasone and/or ether stress exert their effect directly on the midbrain they tend to indicate that this region may play a role in mediating the inhibitory effects of this synthetic steroid on the response to ether stress. This idea is supported by the finding that steroid-sensitive neurones are present in the midbrain, as well as in the hypothalamus (Steiner, Ruf & Akert, 1969).

These findings also emphasize the need for adequate controls when investigations of changes in brain NA function are carried out during, or shortly after, ether anaesthesia.

In summary, the results described here tend to support the theory of NA involvement in the regulation of stress-induced ACTH release. Furthermore, they indicate that the midbrain as well as the hypothalamus may be involved in mediating the inhibitory effects of betamethasone on the response to ether stress. Although direct estimates of CA levels are adequate for a preliminary investigation, more detailed and more localized studies of CA metabolism are also necessary.

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