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HYPOTHALAMIC ADRENALINE SYNTHESIS AFTER STIMULATION OF THE MEDIAL FOREBRAIN BUNDLE

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1 The problem of whether locally released noradrenaline can be methylated to adrenaline in the hypothalamus has been investigated.

2 During stimulation of the medial forebrain bundle (MFB) the hypothalamic adrenaline content increased somewhat, but the increase was not statistically significant (13%, mean of 10 experiments).

3 After inhibition of the activity of monoamine oxidase and catechol-*O*-methyltransferase this increase was much larger (80%, mean of 9 experiments).

4 Adrenalectomy did not prevent the rise in hypothalamic adrenaline after stimulation of the MFB. These results suggest that noradrenaline released during activity of noradrenergic hypothalamic structures may be methylated to adrenaline in the hypothalamus.

Introduction

This paper describes an investigation into whether the noradrenaline released by stimulation of the medial forebrain bundle (MFB) in the hypothalamus (Torda, 1977) is, at least in part, methylated locally to adrenaline.

Methods

Adult male cats were used. Two weeks before the experiment they were anaesthetized with halothane-nitrous oxide and a monopolar platinum microelectrode (0.5–1 μm tip diameter) was lowered into the medial forebrain bundle at A 10.3; L 2.1 and D −3.8 mm stereotaxic coordinates (Snider & Niemer, 1961). A chemitrode was implanted in addition into the ventromedial area of the hypothalamus to allow drugs to be injected. It consisted of a 14.5 mm long 28-gauge hypodermic needle inside a 14 mm long 22-gauge needle (Brown & Fial, 1975; Laroux & Myers, 1975). Electrodes and chemitrodes were fixed to the skull with acrylic cement. Tip positions were ascertained posthumously.

The cats were divided into 4 groups: (1) non-stimulated, non-drug-injected controls; (2) stimulated, non-drug-injected; (3) non-stimulated, drug-injected; (4) stimulated, drug-injected. The drugs injected into groups 3 and 4 were a combination of pyrogallol (4 mg/kg), an inhibitor of catechol-*O*-methyltransferase (COMT), and SU 11739 (Ciba, *N*-methyl-*N*-2-propynyl-1-indamine, 40 mg/kg), an inhibitor of monoamine oxidase (Maitre, 1967). The substances were dissolved in small amounts of 0.9% w/v NaCl solution (saline) and injected into the chemitrode with a Hamilton syringe 50 min before decapitation of the cats. The medial forebrain bundle (MFB) of the cats in groups 2 and 4 was stimulated 20 min before decapitation: rectangular pulses (0.2 ms, 40 Hz, 30–100 nA; 0.15 s pulse sets alternated with 1 s rest periods) were generated by means of a Tektronix Pulse Generator (Series 160, 161, 162). The current was delivered across a stimulus isolation unit to the implanted microelectrode. In order to simulate physiological conditions, stimulus intensities were kept to a minimum. These near-threshold current intensities were sufficient to activate noradrenergic processes, partially because of summation during the 20-min stimulation period.

Assay of noradrenaline and adrenaline

These substances were extracted together from the hypothalamus by the method of Braestrup, Nielsen & Scheel-Kruger (1974), and were assayed fluorimetrically following the method of Compuzano, Wilkerson & Horvath (1975). The threshold of the method was 500 times lower than the smallest amounts of adrenaline found in the hypothalamus.

Table 1 Effect of electrical stimulation of the medial forebrain bundle on the hypothalamic noradrenaline and adrenaline content

		Noradrenaline (NA)				*Adrenaline (A)*					
		Flourimetric method		*Radioenzymatic assay*		*Fluorimetric method*			*Radioenzymatic assay*		
	No. of cats	*(ng per g tissue)*	*(% of NA in control sample)*	*(ng per g tissue)*	*(% of NA in control sample)*	*(ng per g tissue)*	*(% of NA in same sample)*	*(% of A in control samples)*	*(ng per g tissue)*	*(% of NA in same sample)*	*(% of A in control samples)*
Controls	10	1317 ± 11.23	100	1264 ± 16.50	100	149 ± 4.04	11	100	164 ± 5.20	13	100
Stimulated	10	1198 ± 10.03	91	1138 ± 15.99	90	192 ± 5.53	16	128*	204 ± 6.24	18	124*
						Cats injected with pyrogallol and SU 11739					
Controls	8	1386 ± 15.57	100			158 ± 5.93	12	100			
Stimulated	9	1296 ± 16.38	94			285 ± 5.58	22	180*			

Values are mean ± s.e. mean.

* Comparison with unstimulated controls ($P < 0.001$).

The adrenaline and noradrenaline contents of the samples were assayed simultaneously using 410 nm excitation and 505 nm emission wavelength for adrenaline and 395 nm excitation and 485 nm emission wavelength for noradrenaline. All estimations were made in duplicate. The methods of the two groups of authors were followed exactly and the precision of assays could be reproduced. Recoveries of adrenaline and noradrenaline from biological samples were estimated by adding standard solutions of catecholamines (100 ng/ml) containing tracer amounts of tritium labelled adrenaline (from 10 to 500 ng) and ^{14}C-labelled noradrenaline (100 to 1500 ng). Recoveries were in the order of 97%.

Hypothalamic adrenaline and noradrenaline content of cats in groups 1 and 2 (not injected with pyrogallol) were also assayed by the radioenzymatic method of Passon & Peuler (1973). Recoveries of added adrenaline and noradrenaline from tissue extracts reproduced the values obtained by Passon & Peuler with a maximum difference of 2%. As the radioenzymatic assay is based on the enzymatic introduction of an *O*-methyl group into the catecholamine molecule by the action of COMT, it could not be used for tissues from cats treated with the COMT inhibitor.

Materials

All materials were purchased from New England Nuclear Co., Calbiochem, and Fisher Scientific Co.

Adrenalectomy

One week before the acute experiments trans-abdominal adrenalectomy was performed on a group of cats. During the postoperative period the cats were given 5 mg/kg cortisone and 0.5 mg/kg aldosterone daily.

Statistical evaluation

The statistical significance of the observed differences between mean values was tested by Student's *t*-test.

Results

In control cats the hypothalamic noradrenaline content average 1317 ng per g and the adrenaline content 149 ng per g wet weight. Stimulation of the MFB caused an increase in the hypothalamic adrenaline concentration by 24 or 28% ($P < 0.001$) depending on the assay (Table 1). In cats in which the catabolism of noradrenaline and adrenaline by monoamine oxidase and COMT was prevented by the injection of pyrogallol and SU 11739, electrical stimulation of the MFB caused a considerably larger rise (± 80%) in hypothalamic adrenaline than in non-injected cats (see Table 1). In both groups there was a slight but not significant fall in the noradrenaline concentrations. That the rise in the adrenaline content of the hypothalamus after MFB stimulation in the drug-treated cats was not due to uptake from the blood stream of adrenaline released from the adrenal medulla can be inferred from the results obtained on adrenalectomized cats (see Table 2). Adrenalectomy was without effect on the adrenaline concentrations in the hypothalami of the control cats. However, there was still a rise of 78% in the hypothalamic adrenaline concentrations after stimulation of the MFB in those cats which were injected with the enzyme inhibitors.

Discussion

Adrenaline, catecholaminergic neurones and specific phenylethanolamine-*N*-methyltransferases have been located in the same brain regions. The principal

Table 2 Intrahypothalamic synthesis of adrenaline during stimulation of the medial forebrain bundle in adrenalectomized cats

	No. of cats	*Adrenaline content of hypothalamus (ng per g tissue)*	*(% of controls)*
Controls	5	131 ± 11.3	100
Stimulated	5	160 ± 12.5	122
Cats injected with pyrogallol and SU 11739			
Controls	4	139 ± 12.2	100
Stimulated	4	247 ± 13.4*	178*

Values are mean ± s.e. mean.

*Comparison with unstimulated controls ($P < 0.005$).

source of intracerebral adrenaline is methylation of noradrenaline by one of the specific phenylethanolamine-*N*-methyltransferases (Axelrod, 1968; Ciaranello, Barchas, Byers, Stemmle & Barchas, 1969; Pohoreczky, Zigmond, Karlen & Wurtman, 1969; Joh & Goldstein, 1973; Eagles & Iqbal, 1974; Lee, Schultz & Fuller, 1974; Laduron, 1974; Pendleton & Gessner, 1975; Saavedra, Grobecker & Axelrod, 1976; Torda, 1976a, b).

Electrical stimulation of the MFB releases hypothalamic noradrenaline in the cat (Torda, 1977). This noradrenaline is either removed by the blood stream, or by reuptake processes, or it is catabolized (in addition to combining with postsynaptic receptors). In the present work evidence was obtained that part of the noradrenaline released was methylated to adrenaline (Table 1). Inhibition of the two principle enzymes involved in catecholamine catabolism, monoamine oxidase and COMT, increased the rise in hypothalamic adrenaline after stimulation of the MFB from 28 to 80%.

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