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Changes in Rat Brain Norepinephrine Levels and Turnover After Olfactory Bulbectomy

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TONNAER, J. A. D. M., H. RIGTER, D. H. G. VERSTEEG AND V. J. NICKOLSON. *Changes in rat brain norepinephrine levels and turnover after olfactory bulbectomy*. BRAIN RES. BULL. 5(6) 683–686, 1980.—After bilateral olfactory bulbectomy in rats a significant increase of norepinephrine (NE) level in the hypothalamus was found. However, no difference was observed between hypothalamic NE turnover of bulbectomized and sham operated animals. In the amygdaloid cortex the NE level was not affected by bulbectomy. In this area, however, the NE turnover appeared to be decreased after bulbectomy. The latter finding may be related to the deficits in passive avoidance behaviour as found in bulbectomized rats.

Olfactory bulbectomy Norepinephrine Hypothalamus Amygdaloid cortex

VARIOUS attempts have been made to develop new animal models for the detection of potential antidepressants. One of the models which has been studied rather extensively is the olfactory bulbectomized rat. After bulbectomy in rats, behavioural variables such as passive avoidance behaviour are changed and these changes are generally reversed by antidepressant drugs [2, 15, 16].

In preliminary experiments, the effects of bulbectomy on neurotransmitter levels in various brain areas were studied. Since the most prominent bulbectomy-induced change was found in hypothalamic NE levels (unpublished results), the present experiments were done to determine the levels and turnover of NE in rat hypothalamus. In addition, NE levels and turnover in rat amygdaloid cortex were studied because this part of the limbic system is closely connected to the olfactory bulb [1, 10, 11] and may play an important role in passive avoidance behaviour [13].

METHOD

Animals and Operation

Male Wistar (WU-Cpb) rats of about 200 g were anaesthetized with 3% tribromoethanol (Avertine), 1.1 ml/100 g bodyweight IP. The animal's head was fixed in a stereotaxic frame. A midline incision in the skin and periosteum was made. The skin and periosteum were retracted, the skull cleaned and a hole (1.5 mmϕ) was drilled by hand in the skull on the midline, 2 mm frontal to bregma. The olfactory bulbs were cut off with a sharpened needle, just rostral to the frontal lobe, and removed by aspiration. The hole was closed by Spongostan Dental, the wound desinfected by Exoseptolix woundpowder and the skin sutured by two agraves.

Sham operations were performed by almost perforating the skull, the skull and dura were then perforated carefully with a needle, so that bleeding arose beneath the skull.

Not more than six animals were housed in a cage and the experimental animals were separated from the sham operated controls.

Experimental Design

NE levels. Twenty-eight days after surgery the animals were decapitated, the brains dissected and the NE concentrations measured in the hypothalamus and amygdaloid cortex. Decapitation, dissection and extraction of NE were carried out following a randomized block design or a completely randomized design, and NE estimated using a fluorimetric respective radiochemical method.

NE concentrations of bulbectomized animals were averaged, as was done for the sham operated controls and differences in NE-concentrations between bulbectomized animals and sham operated controls were represented as the percentage change. Analysis of variance of logarithmized data (randomized block design) or Wilcoxon-2-tailed test (completely randomized design) were used to analyze data statistically.

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TABLE 1

Concentrations of NE (μg/g) in hypothalamus						
	Method A (fluorimetric)				Method B (radiochemical)	
	Mean	(n)	(m)		Mean ± SEM	(n)
SHAM	2.03	(72)	(6)	SHAM	2.38 ± 0.08	(12)
OB	2.43	(72)	(6)	OB	2.95 ± 0.11	(9)
% change	+20% (+5,+37)*			% change	+24%†	
Concentrations of NE (μg/g) in amygdaloid cortex						
	Method A				Method B	
	Mean	(n)	(m)		Mean ± SEM	(n)
SHAM	0.43	(72)	(6)	SHAM	0.82 ± 0.04	(12)
OB	0.42	(72)	(6)	OB	0.79 ± 0.06	(9)
% change	−3% (−9,+12)*			% change	−4% ns	

OB: olfactory bulbectomized.
SEM: standard error of the mean.
ns: not significant.
n: number of animals.
m: number of experiments.
*95% confidence limits.
†α=0.002 Wilcoxon-2-tailed.

NE turnover. A two by two factorial design, arranged in randomized blocks, was used. On day 28 after surgery bulbectomized and sham operated animals either were injected with α-methyl-p-tyrosine (αMT), 300 mg/kg IP or received a placebo treatment. Three hours after treatment the animals were decapitated, brains were dissected and NE was estimated following the fluorimetric method. Criteria for significance of differences between treatment groups were used following analysis of variance in combination with *t*-tests.

Decapitation and Dissection

The animals were decapitated and brains were inspected for the extent of olfactory bulb removal. Brains of animals which had not been operated according to the criterion, i.e. removing 85–90% of the bulbs, were discarded. Then the brains of animals which had been operated successfully were dissected on an ice-cold plate. Dissection was performed following the method described by Popov *et al.* [9]. The hypothalamus was dissected using a pair of forceps. Then, a transverse cut was made perpendicular to the lower surface of the brain and exactly on the optic chiasm. From the caudal part of the brain the cortex was prepared and the amygdala plus pyriform cortex (hereafter referred to as amygdaloid cortex) were dissected. After weighing the dissected brain parts were frozen and stored at −20°C.

Extraction and Estimation of NE

Fluorimetric method. After thawing the tissue was homogenized in cold HCl-butanol (0.85 ml/100 ml) using a potter-elvehjem glass homogenizer with teflon pestle (1000 rpm, 7 strokes, clearance = 0.125). Per 150 mg wet tissue was, therefore, added: 7 ml HCl-butanol to hypothalamic tissue and 2 ml to tissue of the amygdaloid cortex. The homogenate was centrifuged at 20000 rpm during 10 min (Heraus Christ, Cryofuge 20-3) and NE concentrations were estimated in a 0.8 ml sample of the supernatant, following the method described by Schlumpf *et al.* [12]. NE-standards (2 μg/ml) and blancs (HCl-butanol) passed through the same fluorescence procedure.

Radiochemical method. Hypothalamus (about 35 mg) and amygdaloid cortex (about 100 mg) were homogenized in 1 ml 0.1 N $HClO_4$ (cooled in ice) after thawing, using a potter-elvehjem glass homogenizer with teflon pestle (1000 rpm, 7 strokes, clearance = 0.125). The homogenates were centrifuged (10 min, 20000 rpm) and from the supernatants 0.5 ml was frozen and stored at −20°C, for about two weeks. NE estimations were carried out as described before [14]. Standards (50 ng/ml) and blancs ($HClO_4$) passed through the same procedure as the samples.

RESULTS

NE Levels in Hypothalamus and Amygdaloid Cortex After Bulbectomy

In six independent experiments the NE levels in hypothalamus and amygdaloid cortex were estimated, using the fluorimetric method. The data were analyzed statistically and pooled, yielding the results shown in Table 1, method A. In a separate experiment the NE levels were measured by the radiochemical method, as shown in Table 1, method B.

Using the fluorimetric method, a 20% increase in the NE-level of the hypothalamus was found after bulbectomy. Similarly, a 24% increase was found with the radiochemical method. In contrast, neither method revealed changes in

TABLE 2

CONCENTRATIONS OF NE (μg/g) AFTER αMT TREATMENT

Hypothalamus			Amygdaloid cortex			
	Mean	% Induced depletion		Mean	% Induced depletion	(n)
SHAM placebo	2.26		SHAM placebo	0.56		(8)
OB placebo	2.65		OB placebo	0.54		(7)
SHAM αMT	1.70†	25% (10,38)*	SHAM αMT	0.29†	48% (42,54)*	(8)
OB αMT	1.98†	25% (10,38)*	OB αMT	0.36†	34%‡ (26,42)*	(8)

OB: olfactory bulbectomized.
αMT: α-methyl-p-tyrosine.
*95% confidence limits.
†significantly different from corresponding placebo, $p_2<0.05$.
‡significantly different from SHAM, $p_2<0.01$.

the NE levels of the amygdaloid cortex as a result of bulbectomy.

NE Turnover in Hypothalamus and Amygdaloid Cortex After Bulbectomy

As shown in Table 2 a 25% decrease of NE levels in the hypothalamus was found in both bulbectomized and sham operated animals after treatment with αMT. Following αMT treatment the NE concentration in the amygdaloid cortex of sham operated animals decreased by 48%, whereas bulbectomized animals showed a decrease of 34%. Compared to sham operated animals bulbectomy therefore reduced the αMT-induced NE depletion in the amygdaloid cortex by 29% ($p_2<0.01$).

DISCUSSION

As shown, bilateral olfactory bulbectomy of rats results in an increase of NE level in the hypothalamus, while the level in the amygdaloid cortex is unaffected. In the hypothalamus of bulbectomized rats, the αMT-induced depletion of NE is equivalent to that in sham operated animals. In the amygdaloid cortex, however, the αMT-induced depletion is 29% less in bulbectomized rats, as compared to sham operated animals.

In preliminary experiments we did not observe any change in NE levels in the limbic system of bulbectomized rats, 28 days after surgery. Bulbectomy, however, induced a prominent effect on the hypothalamic NE level. Since the amygdaloid cortex is a part of the limbic system, closely related to the olfactory bulb [1, 10, 11], we measured the NE level in this restricted region in the present study. Both findings presented here, i.e. increase in hypothalamic NE and unaltered NE level in the amygdaloid cortex, are comparable with those of the preliminary study, but in contrast with the results of others [3, 7, 8]. These authors found no changes in hypothalamic NE levels, following bilateral olfactory bulbectomy, but a decrease of NE levels in the pyriform cortex. Telencephalic NE levels, however, are highly dependent on the extent of olfactory system damage [4]. Incomplete removal of the olfactory bulb results in an increase of the NE level, nearly complete removal does not effect the level, while total extirpation leads to NE depletion [4]. Since our surgery resulted in 85–90% removal of the olfactory bulbs, indeed unaltered NE levels in the amygdaloid cortex are expectable, rather than decreased levels. The fact that olfactory bulbectomy results in increased hypothalamic NE levels, whereas there is no effect on hypothalamic NE turnover, cannot be explained and interpreted on the basis of the results obtained thus far.

Up to now biochemical correlates with the olfactory bulbectomy syndrome always have been explained in terms of changes in NE levels [3, 7, 8]. Our studies with αMT in bulbectomized and sham operated rats suggest a decreased NE turnover in the amygdaloid cortex of the bulbectomized rats, as indicated by a decreased depletion of NE after αMT treatment. Since the amygdala plays an important role in passive avoidance behaviour [13], a relation between the reduced noradrenergic metabolism in this area and deficits in passive avoidance behaviour of olfactory bulbectomized rats [2, 15, 16] is possible. Interesting in this respect is the fact that clinically active antidepressants like the tricyclics and like Mianserin are able to normalize this deficit in passive avoidance behaviour [2]. In previous experiments (unpublished) we were unable to observe consistent effects of antidepressants on NE levels in the amygdaloid cortex of olfactory bulbectomized rats. Furthermore, reversal of possible decreased NE levels in the pyriform cortex of bulbectomized animals, by antidepressants, has never been described in literature. It is assumed, however, that these drugs increase noradrenergic activity in the brain either by inhibition of the neuronal re-uptake mechanism (tricyclics) or by an increase of NE turnover (Mianserin [6]). Since our results suggest a decreased NE turnover in the amygdaloid cortex of olfactory bulbectomized rats, it seems worthwhile to elucidate further the role of antidepressants on the NE turnover in this brain region.

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