J. Pharm. Pharmacol. 1980, 32: 630–634 Received January 14, 1980

Effects of olfactory bulbectomy and peripherally-induced anosmia on thermoregulation in the rat: susceptibility to antidepressant type drugs

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Olfactory bulb ablation in the rat produces an acquisition deficit in a step-down passive avoidance test, hyper-reactivity to environmental stimuli, elevated plasma-11-hydroxycorticosterone, and a deficit in the thermoregulatory response to low ambient temperatures, which is not evident at normal ambient temperatures. Like the other features of the bulbectomy syndrome, this thermoregulatory deficit is not related to anosmia per se, since rats made peripherally anosmic with intranasal $ZnSO_4$ (5%) do not show a thermoregulatory deficit at low ambient temperatures. The abnormal response to cold could be prevented by chronic daily administration of amitriptyline (10 mg kg^{-1}), mianserin (10 mg kg^{-1}), and the 5-HT uptake inhibitor, Org 6582 (10 mg kg^{-1}) for 7 days. The possibility that the thermoregulatory deficit has the same biochemical basis as the behavioural changes is discussed and a 5-HT involvement in the syndrome is considered.

Surgical ablation of the olfactory bulbs in rats produces behavioural and biochemical changes defined by an acquisition deficit in a simple passive avoidance test, hyper-reactivity and an elevated circulating 11-hydroxycorticosterone (Cairncross et al 1979). These changes can be reversed by chronic treatment with antidepressant drugs (Cairncross et al 1978a). Since an intrabulbar injection of the neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) also produces an identical syndrome to that of surgical ablation, which is also susceptible to antidepressant drugs (Cairncross et al 1978b), it seems likely that a deficit in a 5-hydroxytryptaminergic system may be the basis of the syndrome. These changes cannot be related to the anosmia (unpublished observation).

Previous reports have suggested that either frontal pole ablation, which includes the olfactory bulbs, or bulbectomy, produced changes in thermoregulation in the rat (Blass 1971; Soderberg & Larsson 1976), but it was not clear whether these changes were due to anosmia or to some other consequence of the loss of the olfactory bulbs.

We have attempted to establish whether precise bulbectomy can induce thermoregulatory deficits in rats subjected to either heat or cold, and if so, whether this was related to the anosmia. Since the other features of the bulbectomy syndrome are susceptible to chronic antidepressant treatment, we tested whether the thermoregulatory changes showed a similar susceptibility as all the features of the bulbectomy syndrome would then have the same biochemical basis, i.e. a functional deficit in a central 5-hydroxytryptamine (5-HT) system.

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MATERIALS AND METHODS

General.—Male Sprague Dawley rats, 200–250 g at the start, were used. Surgical ablation of the olfactory bulbs or sham operation was carried out under Equithesin (3·3 ml kg^{-1}) anaesthesia (as described by Cairncross et al 1978a). Peripheral anosmia was induced by nasal irrigation with 5% Zinc sulphate (w/v) under light ether anaesthesia (Alberts & Galef 1971). Sodium chloride solution (0·9% w/v) was used as a control. Fourteen days after surgery or 24 h after nasal irrigation the temperature responses in the rats to heat and cold were measured.

Animals were also prepared to test for anosmia. They were food deprived for 23 h, placed in a Perspex animal cage (50 × 19 × 13 cm) with the floor covered by a layer of sawdust, 2 cm deep, and the time taken for each rat to locate 4 milk-impregnated food pellets, placed under the sawdust at each corner of the cage was measured. A 5 min cut-off time was used and the results expressed as the cumulative percent of pellets found in each minute of the 5 min.

Thermoregulatory tests. Rats were lightly restrained in Perspex boxes (20 × 12 × 8 cm), core temperature probes were placed 5–6 cm inside the rectum and tail

skin temperature probes were attached securely to the base of the tail. The probes were fed into a YSI telethermometer connected to a potentiometric recorder calibrated for temperature recording.

For the heat load experiments, a rat was placed 65 cm below an infrared lamp (250 W). After a 2 h equilibration period, the lamp was switched on and core and tail skin temperature was recorded for a further 90 min (Cox et al 1976).

A second group of rats were subjected to a cold stress. After an equilibration period of 90 min the rats were placed at an ambient temperature of +4 °C. Core and tail skin temperatures were recorded for 150 min of cold exposure (Cox et al 1979).

Intrabulbar injections. A solution of 5,6-DHT (8 μg in 2 μl of 0·2% ascorbic acid) was slowly infused directly into each olfactory bulb of Equithesin anaesthetized rats over 1 min. These injections were made at co-ordinates 6·0 mm anterior, 2·0 mm lateral and 4·0 mm deep using bregma as the reference point (Pellegrino & Cushman 1967). The injection cannula was left in situ for a further minute to allow diffusion away from the tip. A control group was infused with 2 μl of a 0·2% ascorbic acid solution.

Drug pretreatment. Drugs were injected intraperitoneally daily for 7 days. Treatment began on the 15th post-operative day and continued until the 21st post-operative day. The thermoregulatory responses were determined on the last day of treatment. Drugs were dissolved in 0·9% NaCl (saline) solution.

Drugs used were: amitriptyline hydrochloride (Merck, Sharp and Dohme), mianserin hydrochloride (Organon), Org 6582* (Organon, and 5,6-dihydroxytryptamine creatinine sulphate (Sigma).

Statistics. All results except those for the anosmia test were expressed as mean ± 1 standard error. In all cases the non-parametric Mann Whitney U-test was used as a measure of significance between the groups concerned.

RESULTS

Both olfactory bulbectomized rats and rats receiving intranasal $ZnSO_4$ (5% w/v) failed to locate any of the hidden, milk-impregnated food pellets during the 5 min test period (Table 1). In contrast, sham-operated rats and rats receiving a nasal irrigation with NaCl solution (0·9% w/v) found approximately 50% of the hidden food pellets within the same 5 min period. Thus these results confirmed that both olfactory bulbectomized rats and rats treated intranasally with $ZnSO_4$ had been rendered anosmic.

* ((±)-8-Chloro-11-aminobenzo-(b)-bicyclo-[3.3.1]-nona-3,6a(10a) diene hydrocloride).

Table 1. Effect of olfactory bulbectomy and intranasal $ZnSO_4$ on the ability of rats to locate hidden food pellets as an indication of anosmia.

		Cumulative percent of pellets found in given time (min)				
Procedure	n	1	2	3	4	5
Sham-operation	8	4	13	17	27	43
Olfactory bulbectomy	7	0	0	0	0	0
Instranasal NaCl	8	21	33	58	67	67
Intranasal $ZnSO_4$	8	0	0	0	0	0

At normal ambient temperatures (20 ± 1 °C) there was no significant difference in the mean core temperature or the mean tail skin temperature of any of the groups of rats (Table 2). The thermoregulatory response of sham-operated rats and olfactory bulbectomized rats to a heat load was not significantly different. The mean rise in tail-skin temperature of sham-operates was 3·3 ± 0·8 °C and that of olfactory bulbectomized rats was 3·6 ± 1·4 °C after they had been subjected to the heat load for 60 min. Consequently, core temperature was adequately controlled. The responses of the group receiving either intranasal $ZnSO_4$ or saline was not determined.

The thermoregulatory response of rats exposed to the ambient temperature of +4 °C for 150 min is shown in Fig. 1. The core temperature of sham-operated rats (37·7 °C) fell significantly ($P < 0·01$) more than the controls at +4 °C when recorded after 75 min exposure. The mean total fall in core temperature for bulbectomized rats was 6·2 °C after subjecting them to +4 °C for 150 min.

After chronic amitriptyline pretreatment (10 mg kg^{-1}, i.p. daily, for 7 days) the mean fall in core temperature of the olfactory bulbectomized group maintained at an ambient temperature of +4 °C was 2·5 °C (Fig. 2) which was significantly less than the fall seen in vehicle-pretreated bulbectomized rats (Mann Whitney U-test, $P < 0·01$). Sham-operated rats receiving chronic amitriptyline treatment had a mean fall in core temperature of 4·1 °C when sub-

Table 2. Mean core temperature and mean tail-skin temperature of olfactory bulbectomized rats, sham-operated rats, intranasal $ZnSO_4$-treated rats and intranasal saline-treated rats at normal ambient temperature (20 ± 1 °C).

	n	Core temp. °C	Tail-skin °C
Sham-operated	8	37·5 ± 0·4	27·1 ± 0·6
Olfactory bulbectomy	7	37·8 ± 0·9	27·3 ± 0·6
Intranasal NaCl	8	38·6 ± 0·4	27·1 ± 0·5
Intranasal $ZnSO_4$	8	38·1 ± 0·5	26·8 ± 0·5

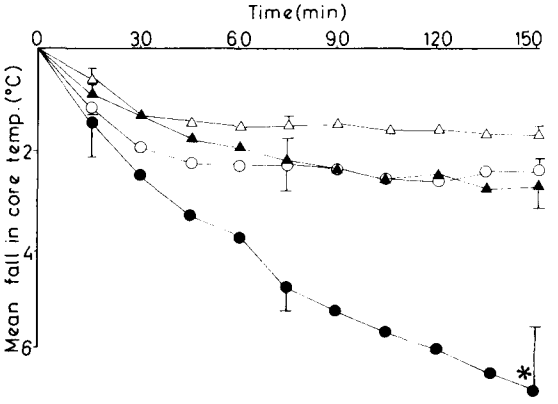

FIG. 1. The mean fall in core temperature (°C) of rats subjected to the ambient temperature of + 4 °C for 150 min. Each point represents the mean of at least 6 results. Standard errors are included at 3 points for clarity, for intranasal sodium chloride solution (△), intranasal $ZnSO_4$ (▲), sham operated (○) and olfactory bulbectomized (●) rats. The non-parametric Mann Whitney U-test was used to determine the level of significance and (*) represents significant difference from sham-operated control group ($P < 0.01$).

jected to an ambient temperature of +4 °C compared with that of 2·7 °C of the saline-treated control sham-operated group. A similar effect on olfactory bulbectomized rats was recorded after either mianserin or Org 6582 (10 mg kg^{-1}, i.p. daily for 7 days) pre-

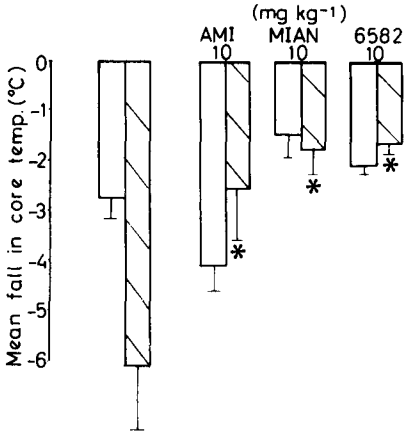

FIG. 2. The effect of chronic treatment with antidepressant drugs on mean maximum fall in core temperature (°C) achieved after rats had been exposed to the ambient temperature of +4 °C for a total of 150 min. Open columns represent sham-operated groups and hatched columns represent olfactory bulbectomized groups. AMI represents amitriptyline (10 mg kg^{-1} i.p. daily for 7 days), MIAN represents mianserin (10 mg kg^{-1} i.p. daily for 7 days) and 6582 represents Org 6582 (10 mg kg^{-1} i.p. daily for 7 days). * represents significant difference from saline-treated bulbectomized group. ($P < 0.01$).

treatment. Thus during cold exposure the fall in core temperature of drug-treated olfactory bulbectomized rats was significantly less than that after saline pretreatment. However, in the case of mianserin and Org 6582 there was no further fall in the core temperature of sham-operated rats (Fig. 2).

Fig. 3 shows the results of intraolfactory bulb injections of vehicle and the neurotoxin, 5–6, dihydroxytryptamine. A similar deficit in thermoregulatory function, at +4 °C ambient, was recorded after intrabulbar 5,6-DHT, to that observed in rats

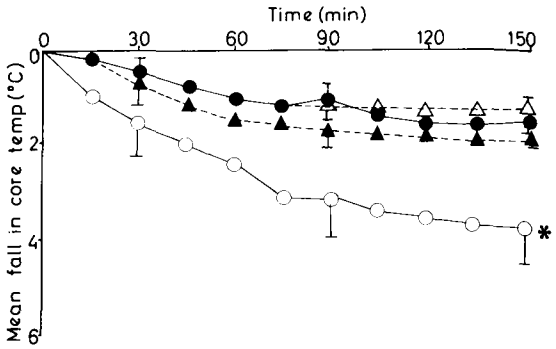

FIG. 3. Shows the mean fall in core temperature (°C) of rats subjected to the ambient temperature of +4 °C for 150 min. Each point represents the mean ± standard error of at least 6 values, where ○ represents rats receiving an intrabulbar injection of 5,6-DHT, ● represents rats receiving intrabulbar vehicle, ▲ represents rats receiving intrabulbar vehicle and chronic amitriptyline (10 mg kg^{-1} i.p. daily for 7 days) and △ represents rats receiving intrabulbar 5,6-DHT and chronic amitriptyline (10 mg kg^{-1} i.p. daily for 7 days). Standard errors are included at 3 points for clarity. The non-parametric Mann Whitney U-test was used to determine the level of significance and * represents significant difference from control vehicle treated group ($P < 0.05$).

with surgical ablation of the olfactory bulbs. The deficit in thermoregulatory response was significantly reversed (Mann Whitney U-test, $P < 0.05$) following chronic treatment of the 5,6-DHT-injected rats with amitriptyline (10 mg kg^{-1}, i.p. daily for 7 days). The fall in core temperature seen in the 5,6-DHT-treated group was significantly greater than that observed in control, vehicle treated rats or vehicle treated rats receiving chronic amitriptyline (Mann Whitney U-test, $P < 0.05$).

DISCUSSION

The results have shown that the olfactory bulbectomized rat possesses an inability to maintain its core temperature when exposed to a low ambient temperature of +4 °C for 150 min. This observation

differs from a previous report which states that a hyperthermic response occurs at low ambient temperatures (Blass 1971). There are two possible reasons for the discrepancy between Blass's results and those of this study. Firstly, our rats were held under only light restraint. Hence although this prevented changes in gross locomotor activity the rats were still able to alter their posture to assist in thermoregulatory control. Blass's (1971) experimental design allowed his animals to move freely. He found that those rats which had been surgically operated possessed a significant hyperthermia and a significant increase in locomotor activity when exposed to low ambient temperatures compared with his control animals. Secondly, Blass (1971) removed a substantial proportion of the brain, including the preoptic region which he termed frontal pole ablation and thus the hyperthermia could have been as a consequence of damage to areas other than the olfactory bulbs.

Soderberg & Larsson (1976) observed thermoregulatory changes of bulbectomized rats and related their findings to the resultant anosmia. In contrast our evidence suggests that although surgical removal of the olfactory bulbs does, as predicted, render the animal anosmic, anosmia per se cannot account for the thermoregulatory deficits. Thus the inability to withstand cold stress must relate to a non-sensory function of the olfactory bulbs. The thermoregulatory deficit at low ambient temperatures was shown to be similar to other features of the bulbectomy syndrome which also could not be related to the anosmia (van Riezen et al 1977). Since the bulbectomy syndrome has been suggested to be a model for detecting drugs with antidepressant potential (Cairncross et al 1978a), it was decided to determine if the abnormal response to cold could be reversed by drugs of the antidepressant group.

Both amitriptyline (a tricyclic antidepressant) and mianserin (a tetracyclic antidepressant) significantly reversed the thermoregulatory deficit of olfactory bulbectomized rats subjected to the low ambient temperature. There was no significant effect on the temperature response of sham-operated rats to the cold exposure when pretreated with any of the antidepressants tested, however, in the case of amitriptyline, the sham-operates were less able to cope with the cold stress, and those treated with mianserin were better equipped to cope with the cold stress. Whatever the reason for this effect in sham-operates it still was not of sufficient magnitude to offset the positive effects observed in bulbectomy. Although only a small number of antidepressants have been tested here, it is suggested that the thermoregulatory deficit of olfactory bulbectomized rats may have the same biochemical basis as the bulbectomy syndrome.

As previous studies have suggested that a 5-HT deficit may underlie the bulbectomy syndrome (Cairncross et al 1978b), two approaches were tried in order to test whether a 5-HT deficit could explain the thermoregulatory changes. Firstly a group of bulbectomized rats were pretreated chronically with the 5-HT uptake inhibitor Org 6582 (Sugrue et al 1976), and in common with the antidepressants this drug reduced the susceptibility of olfactory bulbectomized rats to cold. Thus a 5-HT deficit seems likely since Org 6582 would be expected to increase synaptic 5-HT concentrations. A second and more direct approach was the use of the 5-HT depletor, 5,6-DHT. Cairncross et al (1978b) have recently shown that an intrabulbar injection of 5,6-DHT produces a behavioural syndrome which mimics that of surgical ablation of the olfactory bulbs. Whereas intrabulbar injection of 6-hydroxydopamine or 5,7-dihydroxytryptamine fails to produce such a complete syndrome. Further it has also been shown that chronic antidepressant treatment reverses the 5,6-DHT syndrome (Forster 1978). Since a similar deficit in the thermoregulatory response of rats receiving intrabulbar 5,6-DHT was observed, then this provides further support for a suggestion that a lack of 5-HT is responsible for the thermoregulatory deficit. Finally, chronic antidepressant pretreatment also prevented the thermoregulatory changes observed in 5,6-DHT injected rats, pointing to a further similarity with surgical bulbectomy.

The 5,6-DHT-lesioned animals had a 70% thermoregulatory deficit when compared with rats receiving surgery. This may be due to the fact that after surgical ablation all the 5-HT terminals have been removed whereas after a precise injection with a neurotoxin some neuroterminals in the area survive. Measurement of amine concentrations in various brain regions following neurotoxin injection support this idea (Baumgarten et al 1971), since it was found that 5-HT concentrations were decreased by 70–80%. It has also been reported that 5,6-DHT causes degeneration in dopamine and noradrenaline terminals but to a lesser extent (Baumgarten et al 1974). Therefore the sole involvement of 5-HT in the thermoregulatory deficits may be questioned. However, some functional 5-HT deficit in the whole bulbectomy syndrome seems likely since 6-hydroxydopamine fails to produce such a syndrome.

In conclusion, these experiments have provided evidence for the involvement of 5-HT in controlling

deep core temperature of rats exposed to a cold environment. Other workers have postulated a role for 5-HT in the heat gain pathways of the rat (Myers 1975) and our results are consistent with this view. The precise location of the deficit is uncertain but it seems likely that the origin of the 5-HT neurons concerned is within the midbrain raphé. It is compatible with the present study that stimulation of the raphé has been reported to produce hyperthermia (Sheard & Aghajanian 1967).

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