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Reduction by Propranolol of Raised Urinary Output of MHPG in Hyperactive Rats[1,2]

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SPEISER, Z. AND M. WEINSTOCK. *Reduction by propranolol of raised urinary output of MHPG in hyperactive rats.* PHARMAC. BIOCHEM. BEHAV. 4(5) 531–534, 1976. – Prolonged isolation of rats resulted in hyperactivity in the open field and a significant increase in 24 hr urinary excretion of MHPG (3-methoxy-4-hydroxyphenylglycol). Exploratory activity of group-housed rats in open field was not associated with raised MHPG excretion, compared with that of rats remaining in home cages. Exposure of group-housed rats to 4°C for 2 hr also increased urinary excretion of MHPG. Pretreatment of isolated rats with dl-, d-propranolol or practolol abolished hyperactivity of isolated rats and reduced MHPG output in these rats and in rats exposed to cold. dl-Propranolol did not reduce activity of group-housed rats in open field or their urinary excretion of MHPG. It is suggested that propranolol may have a selective inhibitory effect on stress-induced increases in noradrenaline turnover.

Isolation induced hyperactivity Raised MHPG excretion Inhibition by dl-propranolol d-Propranolol Practolol

A number of attempts have been made to evaluate the role of central noradrenaline in affective disorders by measuring changes in the urinary levels of MHPG (3-methoxy-4-hydroxyphenylethylglycol) [15], the major metabolite of noradrenaline in the central nervous system [10]. Several authors have reported raised levels of MHPG in the urine or cerebrospinal fluid of some manic patients, which returned to normal when the manic phase subsided [7,19]. Furthermore, the increased output of MHPG in these patients was associated with shortened REM (rapid eye movement) sleep times, supporting the view that MHPG excretion may reflect central noradrenergic activity [14].

In a study of the effect of propranolol in acute psychotic patients, it was found that a significant clinical improvement occurred only in those subjects having high pretreatment urinary levels of MHPG [1].

In addition to blocking β adrenoceptors propranolol can also prevent the release of noradrenaline from stimulated sympathetic nerves. This property is shared by the d-isomer, which is a much weaker β adrenoceptor antagonist and by practolol, which lacks local anaesthetic actions [5, 10, 11].

All three drugs selectively reduce the abnormal hyperactivity produced in rats by prolonged social isolation [16]. Their ability to do so may therefore be related to a reduction in an excessive release of noradrenaline rather than to blockade of postsynaptic β adrenoceptors.

If isolation-induced hyperactivity in rats results from an increase in the turnover of central or peripheral noradrenaline, one might expect this to be reflected by an elevated MHPG excretion in the urine. The purpose of the present investigation was to test this hypothesis and to further demonstrate the use of the isolated rat as a model for hyperactivity states involving increased noradrenaline release. Experiments were also designed to see whether the selective tranquilizing effect of propranolol was associated with a reduction in MHPG output.

METHOD

Animals

Male Wistar albino rats were housed in individual cages for 6–8 weeks after weaning, while litter mate controls were housed in groups of 3–5, as previously described [16].

Procedure

Open field test. On the day of the experiment, solitary-housed or group-housed rats were injected subcutaneously with one of the following: saline, 1 ml/kg, dl-propranolol, d-propranolol or practolol, all 1 mg/kg in saline. Fifteen min later the rats were introduced individually into the open field and their motor activity scored for 10 min as previously described as ambulation [16,19].

Immediately after exposure to the open field, the rats

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were transferred to individual metabolism cages, supplied with food and water, and urine was collected for the following 24 hours. Other isolated or group-housed rats were taken directly from their home cages to metabolism cages without exposure to the open field and urine collected as above. In each experiment 4–5 group-housed rats were compared with solitary housed animals given saline and one of the drugs. All experiments were carried out at a constant ambient temperature of 24–25°C, between 900 and 1500 hr. Activity and urine analysis data from appropriate groups were pooled from the individual experiments and were analysed for significant differences between treatments by Student's *t* test.

Cold exposure test. Male Wistar rats, 180–200 g, previously housed in groups of 5, were injected subcutaneously with either saline, 1 ml/kg, or dl-propranolol, 1 mg/kg and placed in individual metabolism cages. These were maintained at an environmental temperature of either 4°C or 24°C for 2 hr. Urine was collected from each rat during these 2 hr and for a subsequent 22 hr when all rats were transferred to an environmental temperature of 24°C. Creatinine concentration was determined in aliquots of all urine samples. The remainder of the urine was acidified with $6NH_2SO_4$ to pH 1 and stored at −20°C until assayed for MHPG content, 1–3 weeks later.

MHPG Estimations

MHPG-sulphate was estimated in all urine samples by a modification of the method of Wolf *et al.* [19].

Five ml aliquots of urine were adjusted to pH 5.2 with 2N NaOH and incubated for 18 hr with 10 units of sulfatase (0.1 ml solution in 60% saturated ammonium sulphate). After ethyl acetate extraction, 4 ml of the supernatant were evaporated to dryness. The residue was dissolved in 0.15 ml ethyl alcohol and 0.1 ml of this solution was chromatographed in two dimensions. After drying and fixing at 100°C, the spots were developed with diazotised paranitroaniline, cut from the paper and eluted with methanol carbonate. The optical density of these solutions was determined at 520 mμ on a Gilford spectrophotometer. Their MHPG content was estimated from a calibration curve provided by density readings of known amounts of MHPG piperazine salt added to 5 ml water and subjected to the same extraction procedure on each occasion that estimations were performed.

Drugs

Drugs used were dl-propranolol hydrochloride, d-propranolol hydrochloride and practolol hydrochloride, kindly supplied by I.C.I. (Macclesfield) Ltd., Sulfatase, (Sigma) and MHPG piperazine salt, (Sigma). All doses are expressed as mg/kg of salt.

RESULTS

As in our previous study, 6–8 weeks of isolation produced a significant increase in the activity of rats in the open field test compared with group-housed controls. One mg each of dl-propranolol, d-propranolol and practolol markedly reduced the hyperactivity of the isolated rats.

In grouped rats however, propranolol caused a slight but significant increase in exploratory activity at this dose level. (See Table 1.) There were no significant differences in the creatinine clearances of either isolated or grouped rats exposed to the open field or remaining in the metabolism cage. The urine output was higher in isolated rats whether they were exposed to the open field or not. The β adrenoceptor blocking agents and d-propranolol reduced the urine volume in the isolated rats but not in the normal animals. (See Table 1.)

There was no difference in the MHPG excretion in isolated or grouped rats which remained in their home cages. However, 10 min in the open field resulted in a marked increase in the output of this metabolite in the urine of isolated rats, while the amount from group-housed animals did not differ from that of animals not exposed to the open field. Pretreatment of isolated rats with either d-, or dl-propranolol or practolol prevented the rise in urinary excretion of MHPG. dl-Propranolol had no effect on the output of MHPG in normal rats exposed to the open field. (See Table 1.)

Exposure of group-housed rats for 2 hr to 4°C, produced no significant change in either the creatinine clearance or urine volume. However, MHPG excretion was significantly

TABLE 1

THE EFFECT OF -BLOCKING AGENTS ON ACTIVITY IN OPEN FIELD TEST AND URINARY EXCRETION OF MHPG

Treatment	No of Rats	Motor Activity Score ±SE	Creatinine mg/24hr/rat ±SE	Urine Vol. ml/24hr/rat ±SE	MHPG μg/24hr/rat ±SE	Level of Significance *p* Motor Activity	MHPG
Isolated rats							
1. Home cage	30	—	8.9 ± 1.9	24.4 ± 2.7	11.4 ± 0.8	—	
2. Open field	45	140 ± 9	11.2 ± 0.9	23.1 ± 2.2	22.0 ± 1.6	—	(1-2)<0.01
3. dl-prop. 1mg/kg	34	76 ± 7	9.0 ± 0.6	15.4 ± 1.8*	13.7 ± 1.7	(2-3)<0.001	(2-3)<0.01
4. d-prop. 1mg/kg	20	77 ± 7	8.9 ± 1.4	15.6 ± 1.4*	14.2 ± 3.0	(2-4)<0.001	(2-4)<0.05
5. practolol 1mg/kg	8	64 ± 14	9.2 ± 1.2	16.0 ± 2.4*	16.4 ± 1.4	(2-5)<0.001	(2-5)<0.05
Grouped rats							
6. Home cage	20	—	8.8 ± 0.1	16.4 ± 2.4	10.8 ± 2.2	—	(1-6)>0.1
7. Open field	27	49.6 ± 5.0	8.3 ± 1.3	11.2 ± 3.2	11.7 ± 2.1	(2-7)<0.001	(2-7)<0.01
8. dl-prop. 1mg/kg	12	72.0 ± 8.0	8.8 ± 0.2	13.5 ± 2.1	12.1 ± 2.1	(7-8)<0.05	(7-8)>0.1

*Significantly different from rats in Group 2. $p<0.05$.

TABLE 2

URINARY EXCRETION OF MHPG AFTER COLD EXPOSURE

Treatment	No of Rats	Creatinine mg/24hr/rat ±SE	Urine Vol. ml/24hr/rat ±SE	MHPG μg/24hr/rat ±SE	Level of Sig. p
1. Saline, 24°C	15	9.6±0.4	18.9±2.0	16.5±1.2	—
2. Saline, 4°C	15	9.9±0.8	19.6±1.6	21.8±2.2	(1-2)<0.01
3. dl-propranolol 1 mg/kg 4°C	10	8.8±0.9	20.5±5.3	13.8±1.3	(2-3)<0.01

elevated compared with that of rats maintained at room temperature (24°C). Pretreatment with 1 mg of dl-propranolol prevented the increase in excretion of MHPG in cold exposed rats. (See Table 2.)

DISCUSSION

There is some evidence that the hyperactivity, induced in rats by prolonged isolation, may be associated with an increase in noradrenaline activity in the central nervous system. Stolk *et al.* [15] showed that social isolation increased the turnover of noradrenaline but not that of serotonin in the brain. Furthermore, the hyperactivity syndrome can be abolished when brain noradrenaline, but not dopamine, is reduced by 40% by intraventricular 6-hydroxydopamine [17].

In the present study the hyperactive behaviour of solitary-housed rats in the open field was accompanied by a significantly increased excretion of MHPG in the urine, compared with such animals which remained in their home cages. Rats which had been raised in groups did not excrete more MHPG than did animals which were not exposed to the novel situation. There was also no difference in the urinary excretion of MHPG in isolated and group-housed rats remaining in their home cages. This suggested that significant differences in the amount of noradrenaline released in solitary-housed and grouped rats only occurred when the animals were exposed to the open field.

There is some evidence that emotional stress can cause a reduction of noradrenaline in the brains of various animal species [2,3], and an increase in the urinary excretion of MHPG in normal human subjects [12]. Thus it is possible that emotional stress results in an increase in the release of noradrenaline in the central nervous system. Exposure of human subjects to cold also results in an elevated urinary excretion of MHPG but not of VMA (vanillylmandelic acid) [4], while cold exposure of rats results in an increase of MHPG in rat brain with no change in noradrenaline levels, indicating increased central noradrenaline turnover [9].

On the other hand, physical exercise alone in normal human subjects [6] or animals [2], not accompanied by emotional stress, does not alter either the level of noradrenaline in the brain [2] or the amount of MHPG excreted in the urine. Urinary output of MHPG was also increased in normal rats in our experiments by exposing them to 4°C under conditions in which motor activity was severely limited by the small size of the container housing them. This suggests that the excretion of raised amounts of MHPG in the urine of isolated rats may not result from the increased motor activity, but is associated with a psychic or emotional stress which is produced in the rats by the novel situation. It therefore appears that solitary confinement, in which there is prolonged deprivation of social contact, makes rats become overactive to situations which are relatively innocuous to animals reared in groups.

Pretreatment of isolated rats with dl-, d-propranolol or practolol reduced both the hyperactivity syndrome and the elevated MHPG excretion. At the same time, activity of group-housed rats in the open field, was, if anything, increased by propranolol and urinary output of MHPG remained unchanged. In normal rats exposed to cold, however, propranolol also reduced MHPG output.

This selective effect of propranolol on abnormal behavior contrasts with the general sedative action of diazepam, which reduced both the hyperactivity of isolated rats and normal exploratory activity at the same dose of 1 mg/kg [17]. This suggests that propranolol acts by inhibiting specifically, a process which increases noradrenaline release in response to either cold exposure or to the stress of a novel environment in susceptible rats.

Our findings also support that of Atsmon *et al.* [1], and suggest that propranolol may have a beneficial effect in psychotic patients with excessive noradrenaline turnover, as indicated by high urinary levels of MHPG.

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