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β-Adrenoceptor Antagonists May Attenuate Hyponeophagia in the Rat Through a Serotonergic Mechanism[1]

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SHEPHARD, R. A., D. A. BUXTON AND P. L. BROADHURST. *β-Adrenoceptor antagonists may attenuate hyponeophagia in the rat through a serotonergic mechanism.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 741–744, 1982.— The unconditioned inhibition of feeding in a novel setting (hyponeophagia) was reduced by propranolol and another potentially centrally acting β-adrenoceptor antagonist, pindolol but not by a peripherally acting one, atenolol. A similar attenuation of hyponeophagia was seen following the 5-HT antagonist methysergide but no consistent effects were observed following some dopamine antagonist drugs. 5-Methoxy N,N-dimethyltryptamine (5-MeODMT), a compound with central 5-HT agonist properties, consistently potentiated hyponeophagia, an effect which was reversed by the centrally acting β-adrenoceptor antagonists and by methysergide. The results are interpreted as evidence for a 5-HT mediation of hyponeophagia and for a probable central 5-HT antagonist role for propranolol and pindolol.

Hyponeophagia Neophobia Serotonin Dopamine β-Adrenoceptor antagonists 5-MeODMT

THERE is accumulating evidence that propranolol and certain other β-adrenoceptor antagonists may also possess significant antagonist activity at 5-hydroxytryptamine (serotonin, 5-HT) receptors. This evidence comes partly from *in vitro* studies in which some β-adrenoceptor antagonists displace (^{3}H) 5-HT from its receptor sites on CNS- derived synaptic membranes [9] and also from work with isolated tissue and ganglion preparations sensitive to 5-HT [3,11].

Some β-adrenoceptor antagonists have been shown to inhibit behavioral syndromes throught to be dependent on raised brain 5-HT activity [4,7]. Iontophoretically applied propranolol inhibits responses of Purkinje fibres in the cerebellum to 5-HT [19].

5-HT receptor antagonists increase punished operant response rates in conflict tests (e.g., [6]) but propranolol appears to be inactive unless given in large doses with chlordiazepoxide [12]. Also, and unlike methysergide, propranolol does not facilitate self-stimulation of the medial fore-brain bundle [18]. However, propranolol elevates food intake suppressed by novelty (hyponeophagia), as do the serotonin antagonist methysergide and benzodiazepines (Shephard and Broadhurst, in preparation). 5-Methoxy-N,N-dimethyltryptamine (5-MeODMT), a proposed 5-HT agonist [2], on the other hand potentiates hyponeophagia ([14] and Shephard and Broadhurst, in preparation) leading us to speculate that propranolol may act as a 5-HT antagonist in this test. In the present study we have further investigated the action of propranolol in reversing hyponeophagia and the effects of 5-MeODMT, to clarify its mechanism of action in this test. Thus effects of propranolol were compared with neuroleptic dopamine antagonist drugs, a mainly peripherally acting β-adrenoceptor antagonist drug, atenolol and another potentially centrally acting β-adrenoceptor antagonist, pindolol. In addition pindolol, but not atenolol, may be expected to have effects at 5-HT receptors as well as at β-adrenoceptor [9].

GENERAL METHOD

Subjects

Male and female, experimentally naive, general strain, hooded rats were used. They were approximately 100 days of age at testing. Prior to testing, subjects were progressively acclimatised to eating from 12.00 to 13.00 BST only. They had free access to food at this time and water at all times except during the test period. Rats were housed three of like sex in metal cages measuring 22 cm×25 cm×20 cm high, and were maintained on an 18 hours light (03.00–21.00 BST) and 6 hours red light, cycle. All rats were bred in the laboratory and reared under carefully standardised conditions.

The 23-hour deprivation schedule was established in 5 days and then maintained for a further 2 weeks before testing. Subjects were randomly assigned by cage to test day and individually randomised for treatment group and order of test within days.

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Apparatus and Procedure

Tests were conducted in an opaque plastic cage of internal dimensions 40 cm×26 cm×13 cm high with a wire top. In the left hand corner distant from the experimenter stood a glass jar 7 cm high and 8 cm diameter with a plastic screw-on lid in which was a central hole of 5 cm diameter. The jar contained standard rat food in powdered form filled to a standard level. The cage stood on the floor in a quiet room with a light intensity of 1775 lux.

The rats were gently placed facing the right hand corner of the cage nearest the experiments. On release a stop-watch was started and after 10 minutes, the subject was removed and returned to its home cage. Three measures were made: (1) Approach latency—the time at which the subject first touched the rim of the jar with a paw or mouth. Latency was recorded to the nearest 0.1 sec. (2) Eating latency—the time at which the subject began its first 3 sec of continuous chewing. To ensure that a significant amount of food was eaten a further criterion was required, the rat had to (a) remain in close contact with the food with its head beneath the rim of the jar, (b) return to the food within a 3 sec period while still apparently eating, or (c) hold some food with its paws while eating. This measure was recorded to the nearest second. (3) Amount eaten—the jar was weighed between each test and as far as possible spilt food was collected and also weighed to calculate the amount eaten. Weighings were made to an accuracy of 10 mg.

Tests were performed between 12.30 and 16.00 BST. The temperature of the test room was between 23°C and 25°C.

Drugs

5-MeODMT (Sigma) was dissolved in 0.9% saline, haloperidol (Janssen), chlorpromazine HCl (Sigma), atenolol (I.C.I.), pindolol (Sandoz), l-propranolol, (I.C.I.), methysergide hydrogen maleinate (Sandoz) were dissolved/suspended in 1% Tween with 0.9% saline. Drug doses were calculated as the salt in each case and all injections were given by the intraperitoneal route (IP), 30 minutes prior to the test.

EXPERIMENT 1

The first study served as a pilot study for the second experiment to establish suitable doses of antagonists and to assess possible effects on eating behaviour. Diazepam attenuates hyponeophagia but with an inverted U-shape dose/response curve (Shephard and Broadhurst, in preparation) and it was thus necessary to determine whether the antagonists in this study demonstrate a similar relationship. In the case of neuroleptic drugs, other studies did not suggest that hyponeophagia would be antagonised [10,15], but in view of the importance of non-specific arousal in hyponeophagia ([13], and Shephard and Broadhurst, in preparation) it was of interest to investigate these drugs in the present procedure. Dose/response relationships for l- and d-propranolol, pindolol, atenolol, methysergide, haloperidol and chlorpromazine were determined for their effects on eating latency.

RESULTS AND DISCUSSION

Results are shown in Fig. 1. Atenolol and d-propranolol failed to affect hyponeophagia in a range of doses from 3–30 mg/kg and are not represented in the figure. Pindolol, methysergide and l-propranolol, however, in low doses produced anti-neophobic effects although methysergide did so over a small dose range. Haloperidol and chlorpromazine

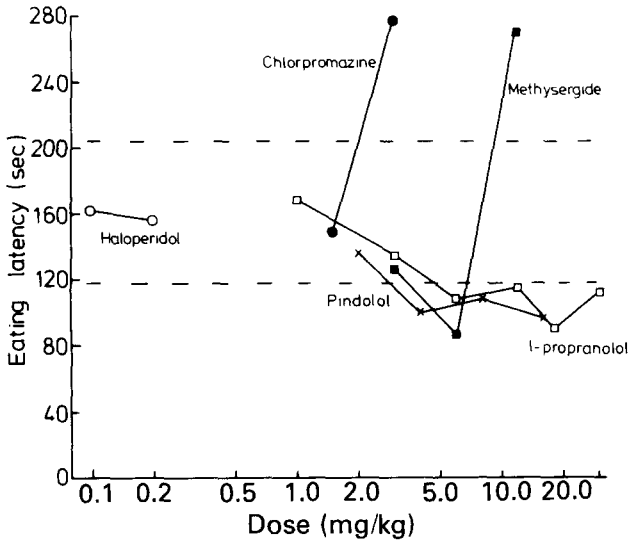

FIG. 1. The effects of haloperidol (open circles) chlorpromazine (filled circles), l-propranolol (open squares), methysergide (filled squares) and pindolol (crosses) on eating latency (ordinate) in male. General strain hooded rats. Dotted lines define the total range of eating latencies observed in 86 experiments on control subjects. Points shown are means of two to five determinations and data from d-propranolol and atenolol are omitted since these drugs showed no activity in a range of doses from 3 to 30 mg/kg. Doses of 0.5 mg/kg haloperidol, of 5 mg/kg chlorpromazine and of 18 mg/kg methysergide caused some subjects tested to fail to eat in the test and latency values for these dosages are consequently omitted.

failed to show anti-neophobic effects and at the highest doses used (0.5 mg/kg and 5 mg/kg, respectively) severely disrupted eating and some rats in each group failed to feed at all during the test period.

EXPERIMENT 2

Following the pilot study a suitable single dose of each antagonist was chosen for a larger study on hyponeophagia using animals of both sexes and examining effects of antagonists on the behaviour alone and when potentiated by a dose of 5-MeODMT.

Few sex effects resulted, and these are discussed elsewhere [13]. Doses were chosen because they either induced anti-neophobic effects in the above study thus: pindolol was used at 4 mg/kg, l-propranolol at 6 mg/kg and methysergide at 6 mg/kg, or were chosen to be the highest dose which did not alone disrupt feeding behaviour, thus haloperidol at 0.2 mg/kg and chlorpromazine at 1.5 mg/kg. Atenolol had no effects in the pilot study but was tested again here at 10 mg/kg. d-Propranolol was not included in this second study but a previous study showed it to be inactive both alone and with 5-MeODMT in this test (Shephard and Broadhurst, in preparation). 5-MeODMT was used in the present study at a dose of 2.5 mg/kg, a dose previously shown significantly to potentiate hyponeophagia in this test ([14] and Shephard and Broadhurst, in preparation). Five rats of each sex, per treatment group were used, in this 7×2×2 design.

RESULTS AND DISCUSSION

Results are shown in Fig. 2 and 3. 5-MeODMT (2.5 mg/kg) significantly prolonged approach latency, $F(1,112)=196.0$, $p<0.001$. None of the antagonist drugs

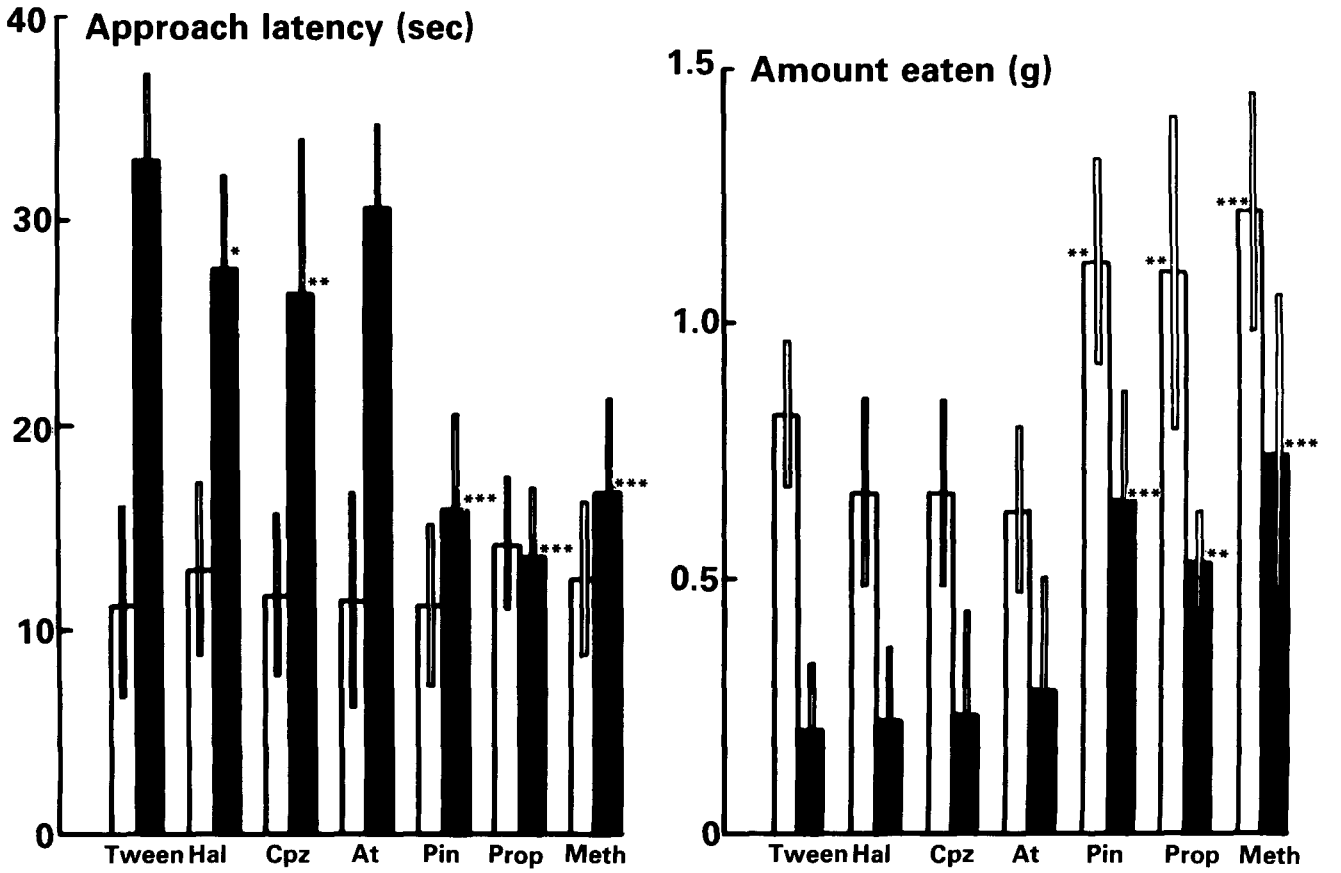

FIG. 2. The effects of drugs on approach latency (left side) and amount eaten (right side) each bar depicting results from five male and five famels rats with an error bar of ±one standard deviation centered on the mean. Hal=haloperidol (0.2 mg/kg), Cpz=Chlorpromazine (1.5 mg/kg), At=atenolol (10 mg/kg), Pin=pindolol (4 mg/kg), Prop=l-propranolol (6 mg/kg) with Meth=methysergide (6 mg/kg). Open bars indicate subjects treated with an additional control (saline) injection, solid bars indicate subjects also receiving 5-MeODMT (2.5 mg/kg). Significance of the differences of the means from the appropriate control means, that is saline only for all open bars and 5-MeODMT only for all solid bars, are indicated by asterisks as follows: *=5% level, **=1% level and ***=0.1% level.

alone modified the approach latency but this may represent a "floor" effect. All the antagonists except atenolol, however, attenuated the effects of 5-MeODMT and a significant interaction in an ANOVA performed on this data was seen, $F(6,112)=14.8, p<0.001$. Although the dopamine antagonists haloperidol and chlorpromazine significantly attenuated the effect of 5-MeODMT the magnitude of the attenuation was not as great as seen following pindolol, propranolol or methysergide (Fig. 2).

The amount eaten was significantly reduced by 5-MeODMT, $F(1,112)=187.0, p<0.001$, and significantly increased by methysergide, pindolol and l-propranolol, $F(6,112)=6.5, p<0.001$, as shown in Fig. 2. There were no significant interactions in an ANOVA on this data suggesting that interactions may be of an additive nature. Haloperidol and chlorpromazine were without effect on amount eaten.

Eating latency was significantly prolonged following 5-MeODMT, $F(1,112)=90.7, p<0.001$, significantly reduced by pindolol, l-propranolol and methysergide, $F(6,112)=26.5$, $p<0.001$, but unaffected by haloperidol, chlorpromazine and atenolol in accordance with the pilot study above (Fig. 3). A significant ANOVA interaction between 5-MeODMT and the antagonists, $F(6,112)=9.0, p<0.001$, resulted from two effects: first, the mean eating latencies for rats given both 5-MeODMT and either pindolol, l-propranolol or methysergide were numerically closer to the antagonist component only of the constituent treatments and, second, chlor-

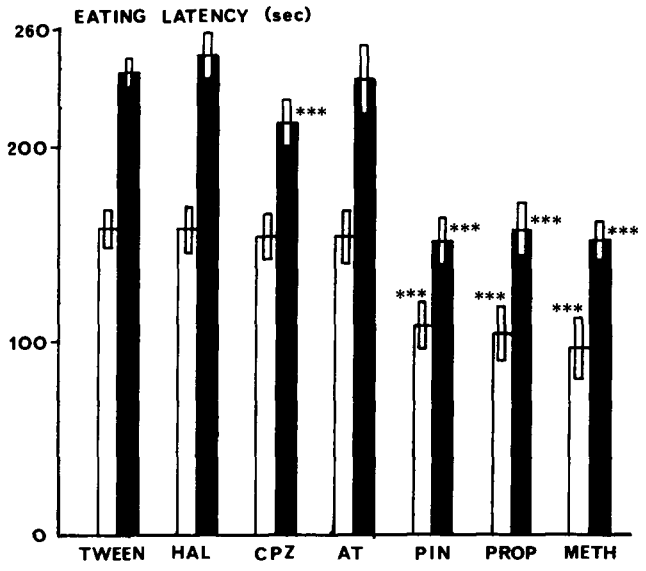

FIG. 3. The effects of drugs on eating latency; each bar depicts results from five male and five female rats with an error bar of ±one standard deviation centered on the mean. Abbreviations and dosage for the drugs used are as in Fig. 2. Significance of the differences from the relevant control means, shown by the first two bars on the left, are indicated by asterisks, ***=0.1% level.

promazine induced a relatively small but significant ($p<0.001$, based on the ANOVA error term) attenuation of 5-MeODMT effects but no effect in the absence of the agonist. Haloperidol and atenolol had no effects.

GENERAL DISCUSSION

The present findings extend previous studies which showed an anti-neophobic effect of the β-adrenoceptor antagonist propranolol (Shephard and Broadhurst, in preparation). This effect is now seen to be shared with another β-adrenoceptor antagonist, pindolol but not with a third, atenolol. Both propranolol and pindolol gain access to the brain following systemic injection whilst atenolol does so only very poorly [16]. Further, propranolol and pindolol, but not atenolol, displace (^{3}H)5-HT from binding sites in rat brain homogenates [9] and there is a growing literature suggesting 5-HT antagonist properties of some β-adrenoceptor antagonists (e.g., [7, 11, 17].

Hyponeophagia appears to have a distinct 5-HT component in that 5-MeODMT potentiates it on three different measures, effects which are antagonised in each case by the 5-HT antagonist methysergide.

Methysergide also attenuates hyponeophagia when not enhanced by 5-MeODMT. These actions of methysergide are also shown by propranolol and pindolol. In the case of attenuation of hyponeophagia, Fig. 1 shows that the maximal effects of these three drugs are similar, suggesting that they act through the same receptors. Benzodiazepines produce greater maximal effect on hyponeophagia (Shephard and Broadhurst, in preparation).

Dopamine antagonists do not appear to consistently attenuate hyponeophagia. Although the approach latency measure enhanced by 5-MeODMT, was attenuated by both neuroleptics, the magnitude of these attenuations were less than those produced by the putative 5-HT antagonist drugs (see Fig. 2).

Since neuroleptics do not antagonise hyponeophagia when given alone, a part of the enhancement caused by 5-MeODMT may be due to its having some dopamine agonist activity. Chlorpromazine attenuated 5-MeODMT effects on eating latency but is known to have effects at non-dopaminergic sites, including 5-HT receptors [1], and therefore this effect of chlorpromazine could be partly due to antagonist effects at 5-HT receptors. Haloperidol, however, is reportedly without effect at serotonin receptors in moderate concentrations [1] and does not attenuate hyponeophagia or affect 5-MeODMT action in this test.

It is reasonable to exclude blockade of dopamine receptors as a possible mechanism for the effects of pindolol and propranolol in these experiments. Firstly, the attenuation of neophobia seen with these drugs was greater in each case than with the known potent dopamine antagonists used in these experiments. Secondly, the effects of propranolol seen here are highly stereospecific for the l-isomer whilst the rare examples of apparent dopamine antagonist effects of this compound show no stereospecificity against dopamine mediated behaviours [8] or show greater potency for the d-isomer [5]. The inactivity of the largely peripherally-acting atenolol suggests that peripheral mechanisms related to β-adrenoceptor blockade are unlikely to be relevant to the effects of pindolol and propranolol reported here. A mechanism involving antagonism at brain β-adrenoceptors cannot be completely ruled out although the lack of β-receptor blocking activity of methysergide makes this unlikely.

The likelihood is, therefore, that the anti-neophobic effects of pindolol and propranolol and their antagonism of 5-MeODMT action are mediated through an antagonism at central 5-HT receptors.

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