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Activation of α_2-Adrenoceptors Suppresses Excessive Wheel Running in the Semistarvation-Induced Hyperactive Rat

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WILCKENS, T., U. SCHWEIGER AND K. M. PIRKE. *Activation of α_2-adrenoceptors suppresses excessive wheel running in the semistarvation-induced hyperactive rat.* PHARMACOL BIOCHEM BEHAV 43(3) 733–738, 1992.—Male Wistar rats were housed in cages linked to running wheels and fed on a schedule designed to reduce their body weight by 20–30%. During this period of semistarvation, rats increased their daily running wheel activity (RWA) by up to 30 km/day. RWA could be kept at this level provided that body weight was kept constant. Different α-adrenergic agonists and antagonists were tested for their effects on RWA and it was found that RWA could be suppressed only by agonists that display high affinity for the α_2-receptor (clonidine and guanfacine). Neither antagonist had an effect on RWA. Clonidine's inhibiting effect on RWA was prevented by pretreatment with yohimbine, which also has high affinity for α_2-receptors. From these results, we conclude that semistarvation-induced hyperactivity can be blocked by α_2-agonists. In view of this result and those that were obtained in previous studies, a theoretical model for the development of semistarvation-induced hyperactivity will be presented.

Clonidine Exercise Anorexia Stress α_2-receptors α_2-adrenoceptors Subtypes Food intake Starvation

FOOD-restricted rats develop excessive running wheel activity (RWA) when given free access to a running wheel. Under certain experimental conditions, hyperactive animals will reduce food intake even further, become anorectic, and eventually die (6,17). These observations are of special interest because hyperactivity occurs in many patients with anorexia nervosa (11). On the basis of studies in animals and on observation in men, excessive physical activity is now thought to play a crucial role in the development and maintenance of this eating disorder (4,5).

Norepinephrine (NE) is known to play a major role in various neuroendocrine and behavioral functions, for example, activation of the hypothalamo-hypophyseal-adrenal (HPA) axis (20), feeding (12), and locomotion (14). In a recent study, its involvement in the control of circadian activity patterns has been characterized (16).

In the brains of exercised male rates, levels of NE and methoxyhydroxyphenylglycol (MHPG) were found to be increased (3). In the rat model of semistarvation-induced hyperactivity, we observed significantly increased NE and MHPG in the hypothalamus with a circadian rhythm parallel to the resting activity cycle when rats were decapitated after 10 days of restricted feeding (1).

The observed influence of starvation and hyperactivity upon the noradrenergic system in the rat may present an interesting animal model for anorexia nervosa because anorectic patients show reduced food intake, hyperactivity, and an altered noradrenergic activity (7,11).

In the present study, we evaluated the effects of α-adrenoceptor agonists and antagonists on RWA in the rat model for semistarvation-induced hyperactivity, where RWA was stabilized for 6 weeks at a high level (ca. 20–25 km/day).

METHOD

Animals

Forty-eight male Wistar rats (200–220 g) (obtained from INTERFAUNA Süddeutsche Versuchstierfarm GmbH & Co., Tuttlingen, FRG) were allowed to acclimatize in the new environment for 10 days (room temperature 25°C, relative humidity 50% and a 12 L : 12 D schedule, with dark beginning at 11:00 a.m.); rats were housed in groups of six in standard plastic cages. Food (Altromin C 1010) and water were constantly available.

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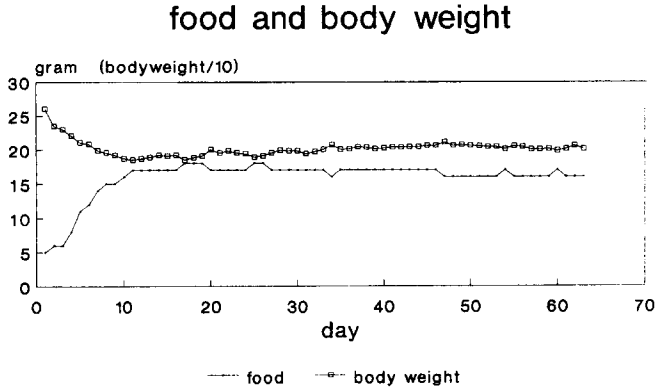
Semistarvation-Induced Running

The following experimental design is a modification of the model for "voluntary prolonged running" (19). On the first day of the experiment, rats were placed in standard running wheel cages with attached home cage compartments (LaFayette Instruments, Lafayette, IN; circumference 1.10 m); throughout the experiment, access to the wheel was free. Rats were weighed daily before the dark phase and food was provided only once in the middle of the dark phase (at 5:00 p.m.).

The following feeding schedule was designed to induce a *stable and reliable state of increased RWA* by the reduction of body weight by 30%: During the first week, rats were fed with increasing amounts of food (6–10 g) to keep weight loss between 5 and 8 g per day; running increased rapidly once their initial body weight was reduced to about 80%. The amount of food was then adapted to their individual RWA and weight loss by slowly increasing the food to 15–17 g/day to prevent weight loss over the targeted 30%. After 3 weeks, a high level of RWA was established and rats needed 30–40% more food than sedentary controls to maintain their body weight constant. Approximately 80% of daily running takes place during the dark period prior to feeding, with its highest intensity occuring in the 2 h that precede feeding. After 3 weeks, rats showed no significant day-to-day variability in RWA (for a representative example of one rat, see Fig. 1).



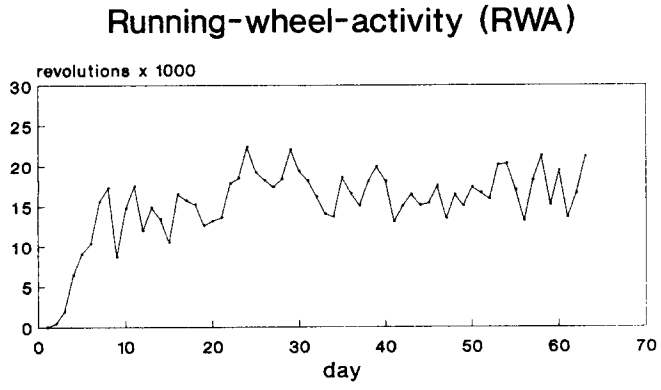
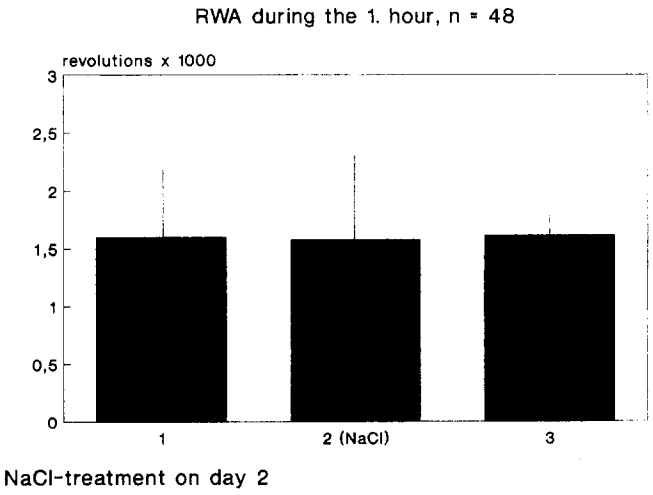


FIG. 1. Upper: Food intake (—●—) and body weight (—⊟—). Lower: RWA in one rat.



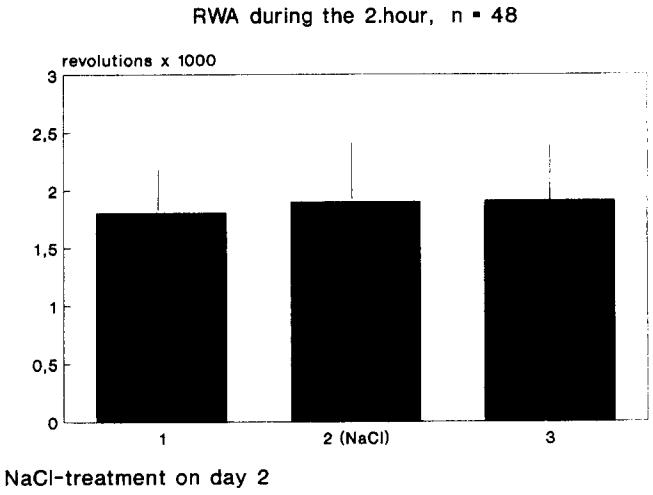


FIG. 2. RWA on 3 consecutive days in 48 rats. On the second day, physiological NaCl was injected. Upper: RWA during the first hour. Lower: RWA during the second hour.

Experimental Design

To avoid the effects of individual differences or seasonal influence on RWA, we used each rat as its own control.

Rats' RWA was recorded over a 24-h period (i.e., one dark phase and one light phase) to ascertain their daily level of activity. The experiment was started at the onset of a dark phase (T = 0 h) and the behavior monitored during the first 4 h to check that it conformed to the established daily activity. At T = 4 h, the appropriate drug was administered. RWA was measured during the 2 consecutive h (T = 5, 6 h) and at T = 6 h rats were fed. The effects of the drugs on RWA

were compared with each individual's activity occurring on the previous day during the latter 2 h without treatment.

Drug Treatment and Dosage

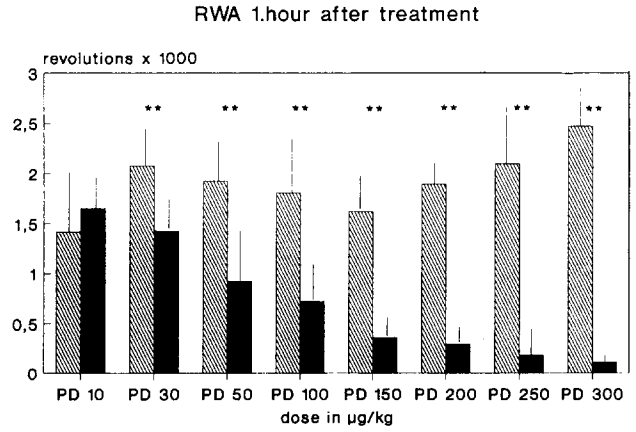
For each drug and each dose, a group of nine rats was treated, *the same group for each dose-response curve.* For combinations between *agonists and antagonists, the same rats were used for each antagonist.*

All dosages were chosen on the basis of previous studies showing marked effects of these on both food intake and locomotion, and are given in mg/kg.

As rats were used repeatedly, there was an interval between treatments of at least 4 days or longer if appropriate.

Drugs

Clonidine, prazosin, phentalomine, yohimbine, propranolol (all Sigma Chemicals, München, Germany), St 586 (Boehringer Ingelheim, Ingelheim, Germany), cirazoline (Synthelabo Recherche, L.E.R.S., Paris, France), and guanfacine (Sandoz Ltd., Basel, Switzerland) were dissolved in 0.9% saline and injected IP.



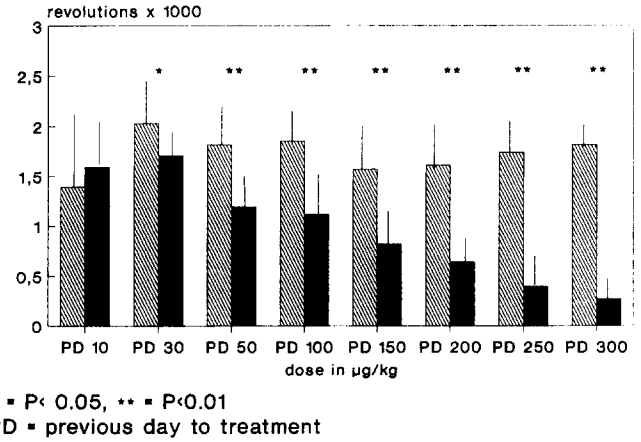
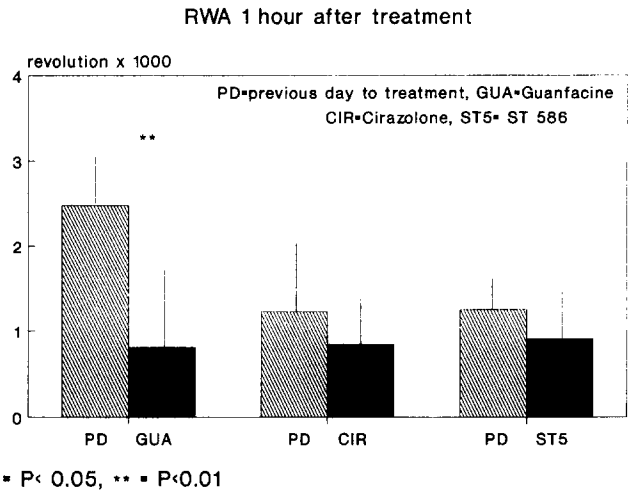


FIG. 3. Dose-response curve of the effect of clonidine on RWA during the first hour (upper) and the second hour (lower) after treatment. $*p < 0.05$, $**p < 0.01$.



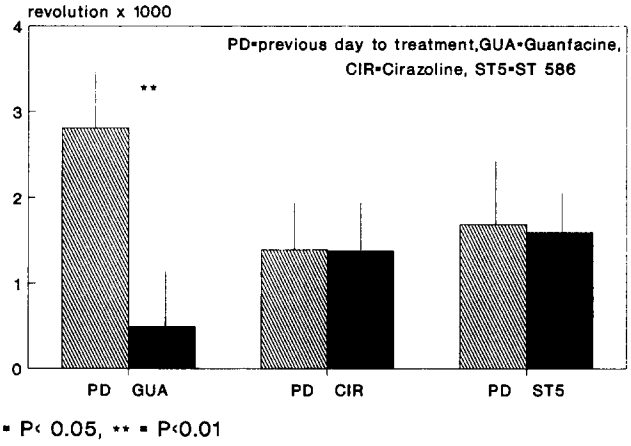


FIG. 4. Effect of α-agonists on RWA during the first (upper) and the second (lower) hour after treatment. Solid column: Treatment; striped column: No treatment (previous day). $*p < 0.05$, $**p < 0.01$.

Statistical Analysis

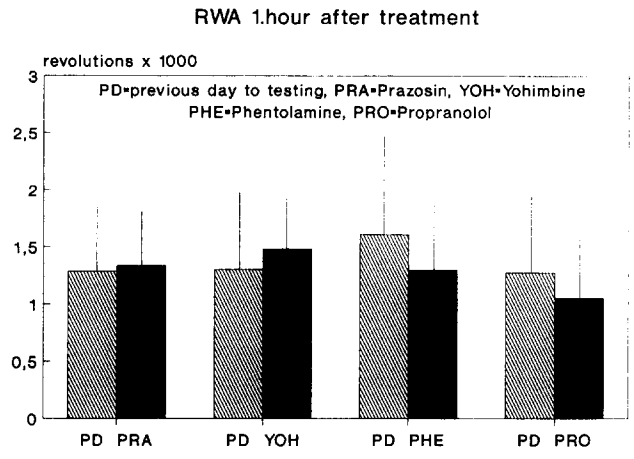
All results are expressed as mean + SD.

Dose-Response Curves

The effects on RWA with (TR = treatment) and without (PD = previous day to treatment) drug treatment were analyzed by a multiple analysis of variance for repeated measures (two-factor MANOVA) and followed by Student's *t*-test for paired data if appropriate. The factors were TR vs. PD and dose.

Effects of Antagonists on Agonists

Groups for agonist and agonist + antagonist were not identical. Therefore, the statistical design was chosen as fol-



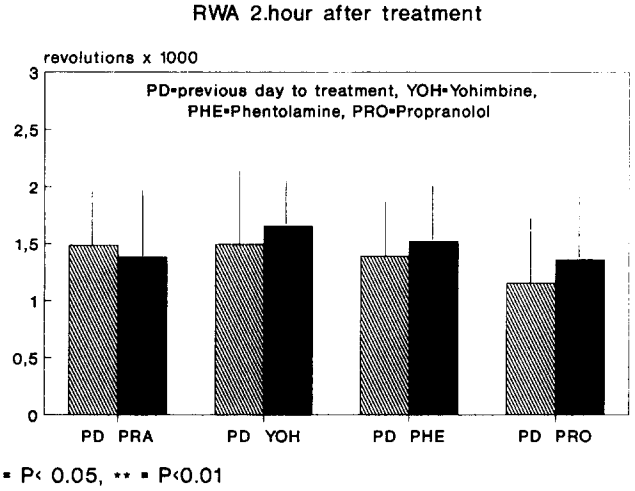


FIG. 5. Effect of noradrenergic antagonists on RWA. Data are presented as in Fig. 4. $*p < 0.05$, $**p < 0.01$.

lows. Comparison between agonist and agonist + antagonist groups were analyzed by a two-factor MANOVA for repeated measures with the factors TR vs. PD and group. A paired *t*-test (TR/PD) was always performed to assess the efficiency of antagonism.

Other Agonists

For drugs of which only one dose was injected, the effect on RWA was assessed by the *t*-test for paired samples.

RESULTS

RWA Within 3 Consecutive Days

Individuals' RWA ($n = 48$) showed no significant differences within 3 consecutive days; NaCl treatment had no influence on RWA (Fig. 2).

Effects of Noradrenergic Agonists on RWA: Clonidine (Fig. 3)

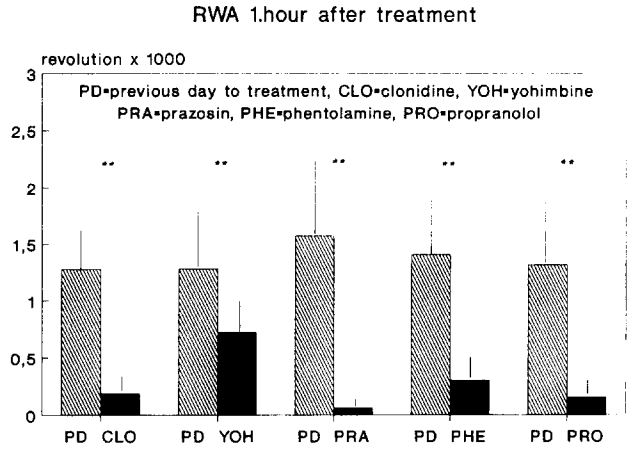
The MANOVA showed a significant effect during both hours of testing, $F(2, 7) = 82.4$, $p < 0.05$, and $F(2, 7) = 51.9$, $p < 0.05$. With increasing dosage of clonidine, the suppression of RWA became more prominent. The *t*-test comparing RWA on previous day vs. RWA on the day of treatment revealed a significant effect on RWA of a dose of 30 μg/kg; during the first hour after treatment, $p < 0.05$; during the second hour, $p < 0.01$. Doses of 50 μg/kg and greater significantly affected RWA ($p < 0.01$).

Other α-Adrenergic Agonists (Fig. 4)

Excessive RWA was also suppressed by the selective α_2-agonist guanfacine (2.5 mg/kg; $p < 0.01$), whereas the α-1-agonists St 586 (1 mg/kg) and cirazolin (0.1 mg/kg) had no effects.

Noradrenergic Antagonists (Fig. 5)

None of the α-adrenergic antagonists, such as prazosin ($\alpha_{1/2b}$, 3 mg/kg), yohimbine (α_2, 10.0 mg/kg), phentolamine ($\alpha_{1/2}$, 10 mg/kg), and the β-adrenergic antagonist propranolol (10 mg/kg), had any effect on RWA when injected alone.



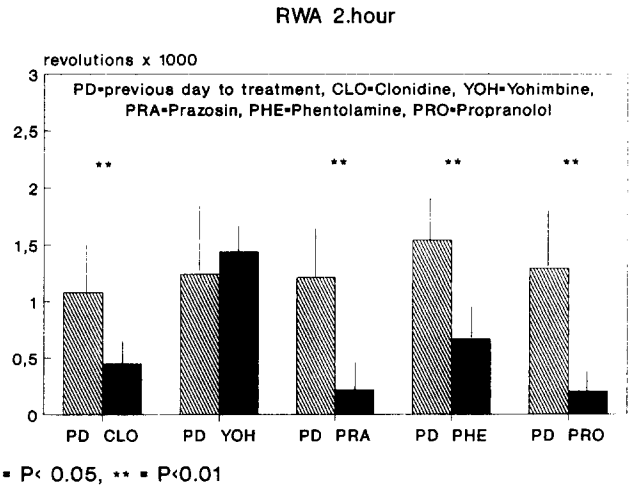


FIG. 6. Effect of noradrenergic antagonists on clonidine. One hour after treatment. Two hours after treatment.

Clonidine and Antagonists (Fig. 6)

During the first and second hours of testing, only the α_2-antagonist yohimbine (α_2, 10 mg/kg) restored RWA suppressed by clonidine [0.3 mg/kg, $F(1, 16) = 5.14$, $p < 0.05$, and $F(1, 16) = 8.04$, $p < 0.05$]; the paired t-test showed that yohimbine antagonized clonidine completely only during the second hour. Prazosin ($\alpha_{1/2b}$, 3 mg/kg), phentolamine ($\alpha_{1/2}$, 10 mg/kg), and propranolol (β, 10 mg/kg) failed to restore RWA.

DISCUSSION

Clonidine and guanfacine suppressed semistarvation-induced RWA. The effect of clonidine was dose dependent. This would indicate that the excessive running in the semistarved rat is suppressed by activation of α_2-receptors, as both compounds display high affinities to the α_2-receptor (21). This is further supported by the fact that none of the α_1-selective agonists, ST 856 and cirazoline (13,18), had any effect on rats' behavior in this experimental paradigm. In addition, the sedative effect of clonidine was antagonized by the α_2-antagonist yohimbine only.

While preparing this article, a study was published describing guanfacine as an α_{2A}-selective agonist (24). In view of this and of the result that clonidine's effect on RWA could not be blocked by prazosin, which displays high affinity to the α_{2B}-receptor (2), it is tempting to speculate that clonidine suppresses RWA by an action on α_{2A}-receptors. However, other α-adrenoceptor subtypes, namely, the α_{2C}- and α_{2D}-subtype, have already been described (2). Furthermore, the unified hypothesis of feedback modulation of NE release has been questioned, as two stereochemically different α_2-receptors seem to be involved in central NE release (8). More selective tools would have been needed to attribute the RWA-suppressive effect of α_{2H}-agonists to a specific receptor subtype.

From the present results, it is not possible to differentiate whether suppression of RWA by clonidine is mediated via central or peripheral receptors. However, it seems unlikely that suppression of RWA is simply a result of a depressed blood pressure, as neither prazosin nor phentolamine, which are as well known for the latter effect (13), had any effect on RWA.

Semistarvation-induced RWA has been shown to be accompanied by an increased NE turnover (1). Therefore, one likely explanation for clonidine's suppression of hyperlocomotion is that it reduces central NE turnover, thus decreasing the stimulation of postsynaptic α-receptors. This would reduce the drive for feeding mediated via postsynaptic α_2-receptors (12). Such an interpretation would be in line with reports that demonstrated clonidine-induced sedation to be a result of stimulation of somatodentric autoreceptors at ascending noradrenergic neurons (10,21).

For clonidine-induced sedation, an interaction with other neurotransmittersystems like the GABA- or serotonergic (5-HT) system cannot be excluded. Thus, when clonidine was injected into the paraventricular nucleus (PVN) the induced sedation was at least in part a result of the influence on the GABAergic system (15). In addition, it has been demonstrated that clonidine-induced sedation depends upon an intact serotonergic system (19,23). The authors of one of these studies, however, suggested this interaction to be of minor importance under physiological conditions without pharmacological manipulations (9).

In another study, it was demonstrated that the increased corticosterone secretion after pharmacologically increased activity of the serotonergic system was partially inhibited by clonidine. This neuroendocrine response was completely blocked by pretreatment with clonidine and cyproheptadine, a 5-$HT_{1C/2}$ antagonist (22). These observations might be important in view of the result obtained in a previous study, where RWA could be suppressed by 5-HT_{1C} agonists (Wilckens' unpublished observation).

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