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Anorectic Effect of Alpha$_2$-Antagonists in Dog: Effect of Acute and Chronic Treatment

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BERLAN, M., J. GALITZKY, M.-A. TRAN AND P. MONTASTRUC. *Anorectic effect of alpha$_2$-antagonists in dog: Effect of acute and chronic treatment.* PHARMACOL BIOCHEM BEHAV **39**(2) 313–320, 1991.—Acute oral administration of alpha$_2$-antagonists (yohimbine, RX 821002, atipamezole: 1 mg/kg each) reduced dog food intake. Yohimbine reduced food intake over 20 hours, while the effect of the two other drugs lasted only 2 hours. Yohimbine (0.4 or 1 μg/kg) gave the same results. At these doses, it promoted a lasting durable increase in plasma nonesterified fatty acids and catecholamines levels and a transient elevation of plasma insulin levels. The beta-antagonist nadolol (4 mg/kg per os) suppressed the yohimbine-induced lipid mobilization without modifying its anorectic effect. Chronic oral yohimbine (0.4 mg/kg/day during 14 days) reduced food intake and promoted a weight loss. Normal food intake was recovered two days after yohimbine withdrawal. No change was observed in the number of platelet alpha$_2$-adrenergic receptors. In addition to their lipid mobilizing action and sympathetic tone stimulation, alpha$_2$-antagonist compounds reduce food intake.

Alpha$_2$-antagonists Yohimbine Food intake Acute and chronic administration Dog Lipomobilization Catecholamines

THE brain controls food intake by various mechanisms which signal satiety and appetite. The reduction of food intake is either a result of a decrease of the desire for food or an increase in the sensation of satiety (13). The main drugs so far identified and studied to a fair extent are those acting on catecholamine or serotonin receptors (12, 29, 30). In the first group, a distinction should be made between amphetamine-like agents which increase central catecholamine turnover and agents that act as beta-adrenergic or dopamine agonists (24,25). The lateral hypothalamus and the perifornical area contain dopaminergic and beta-adrenergic receptors (mainly beta$_2$-adrenergic receptors), the activation of which reduces food intake (27). The role of dopamine in the central control of food intake is more complex and controversial, since a reduction of eating can be easily inhibited with agents that either mimic or antagonize the action of dopamine. Moreover, the modifications of eating, by agents acting on dopamine receptors, are also related to changes in sensory motor mechanisms (38).

The medial hypothalamus (medial region of the paraventricular nucleus) is considered as the satiety center, the neurones of which inhibit eating desire. Its destruction produces overeating and obesity in various animal species (17). It receives noradrenergic and serotoninergic neuronal projections. When injected with norepinephrine, it causes overeating without other major behavioural changes in rats, suggesting a specific action of the neuromediator on feeding (5, 26, 28). Goldman et al. (16) demonstrated that this orectic effect of norepinephrine (mimicked by clonidine, a selective alpha$_2$-adrenoceptor agonist) was due to the stimulation of alpha$_2$-noradrenergic receptors located on the paraventricular nucleus. Although biochemical evidence suggested that alpha$_2$-adrenoceptors are located presynaptically, recent data support the fact that they are present in postsynaptic membranes in the paraventricular nucleus (16,48). Other work indicates that the alpha$_2$-agonist clonidine injected by the peripheral route can increase food intake in the mouse (7), monkey (41), rat (1) and dog (23).

On the other hand, acute administration of yohimbine (an alpha$_2$-adrenergic antagonist that penetrates the central nervous system) suggested the existence of a tonic alpha$_2$-adrenergic inhibition of the central noradrenergic system and the sympathetic nervous system in dogs and humans. In dogs, the infusion of yohimbine and various alpha$_2$-adrenoceptor antagonists induces an increase in plasma norepinephrine and epinephrine levels and, as a consequence, increases the level of plasma nonesterified fatty acids (NEFA) (43,45) and insulin secretion (37,43). These two endocrino-metabolic effects were attributed to beta stimulation on adipose tissue and on beta pancreatic cells, respectively. In man, oral or intravenous yohimbine enhances norepinephrine levels in plasma (11, 18, 36) and also in cerebrospinal fluid (36). So the fact that alpha$_2$-adrenoceptor antagonists activate the central nervous system and the sympathetic nervous system argues for the existence of a tonic alpha$_2$ central inhibition. Taken together, these results suggest that alpha$_2$-antagonists are putative agents for the reduction of food intake.

So the aim of the present study was to test the hypothesis that the paraventricular alpha$_2$-adrenergic system (which stimulates food intake by inhibition of the neurone activity of this system) is tonic in nature by measuring the effect of administration of alpha$_2$-adrenoceptor antagonists on food intake in the dog. The following experiments were undertaken 1) to compare the relative potency of an older (yohimbine) and of the recently developed alpha$_2$-antagonist compounds: RX821002 (21,42) and

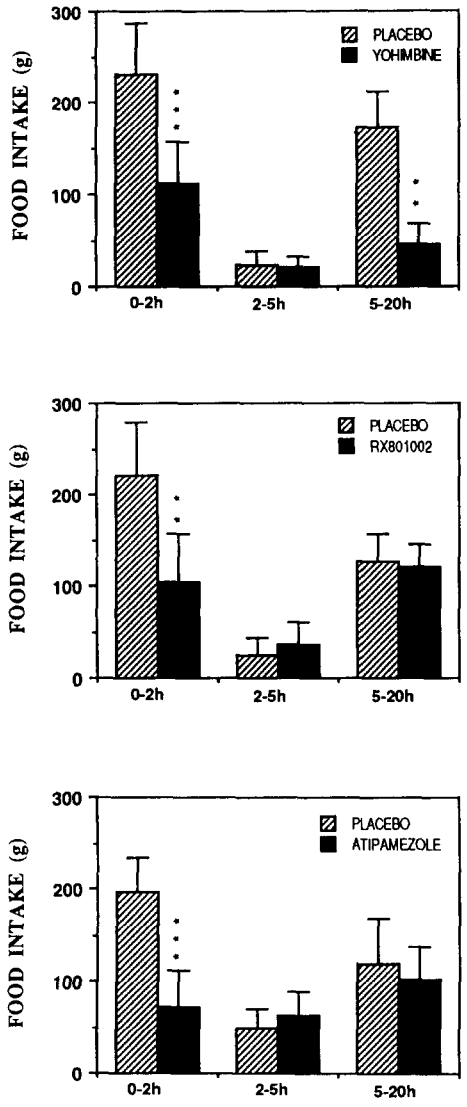

FIG. 1. Effect of acute oral administration of placebo, yohimbine, RX821002 and atipamezole (1 mg/kg) on food intake in 8 dogs. The various alpha$_2$-adrenergic antagonists were administered at 9 a.m., and the dogs had access to food at 12 p.m. Food intake was measured at 2 p.m. (0–2 h, first period), 5 p.m. (2–5 h, second period) and 8 a.m. the following day (5–20 h, third period). Values are means ± S.E.M. $^{**}p<0.02$ and $^{***}p<0.01$ when compared to placebo values.

atipamezole (40,47) on food intake after their acute oral administration, 2) to define the metabolic and endocrinological impact of acute oral administration of alpha$_2$-antagonists and 3) to evaluate the effect of their chronic administration on certain endocrino-metabolic parameters, food intake and body weight.

METHOD

Animals

Eight adult male mongrel dogs (13–29 kg) were used for the study in various experimental situations. They were housed in individual cages (0.8 × 1 × 1 m) with ad lib water access at a constant temperature of 21°C and fed with a common laboratory diet (UAR Company, France, composed of: 10% water, 23% proteins, 4% lipids, 51% glucides, 3% cellulose, vitamin A (10,000 UI/kg) and D (3,000 UI/kg) and various mineral salts). For all protocols, they had free access to food from 12 p.m. to 8 a.m. the following day. Then, from 8 a.m. to 12 p.m., the dogs had access to a park (no food available) and then were returned to their cages at 12 p.m. (food available). In all the studies, dogs were randomly assigned to either drug or placebo treatment. The drug (various alpha$_2$-adrenergic antagonists, nadolol or placebo) was always given orally at 9 a.m., in a capsule included in a small quantity (10 g) of minced meat. A verification of the ingestion was made.

The acute effect of various alpha$_2$-adrenoceptor antagonists on food intake in the dogs was evaluated according to the method of Le Douarec (22) with modification: food consumption was measured from 12 p.m. to 2 p.m. (1st period), from 2 p.m. to 5 p.m. (2nd period) and then from 5 p.m. until 8 a.m. the following day (3rd period). There was a washout period of at least one week between two assays studying the various alpha$_2$-adrenoceptor antagonists.

The effect of yohimbine on food intake was also studied in dogs after pretreatment with the beta antagonist nadolol. Nadolol (4 mg/kg) was administered on the first day (at 9 a.m.) and then at 9 a.m. the following day with 1 mg/kg yohimbine. Dogs had access to food from 12 p.m. until 8 a.m.

During chronic administration of yohimbine (0.4 mg/day per os at 9 a.m.), food intake was measured every day (dogs had free access to food over the period from 12 p.m. to 8 a.m. the following day). Daily food intake for the previous day was calculated by subtracting the weight of the food remaining at 8 a.m. from the amount of food available to the dog on the previous day at 12 p.m. In these experiments, the stability of the body weight was checked and the spontaneous food intake measured over 7 days, then the treatment with yohimbine began for 14 successive days. After that, the treatment was stopped, the dogs weighed and food intake measured during the 14 following days, and the dogs were weighed again the last day. The day before and the day following the end of the treatment, blood samples were taken for measuring plasma catecholamine, glucose, insulin and NEFA levels.

The impact of yohimbine (0.4 mg/kg per os at 9 a.m.) intake on plasma concentrations of NEFA, catecholamines, insulin and glucose was also studied in 8 dogs after an overnight fast. Yohimbine (or placebo) was given orally, immediately after the first blood sample. Blood samples were taken 30, 60, 120, 180 and 240 min after yohimbine (or placebo) administration.

Plasma Determinations

Blood glucose and insulin were determined with a glucose oxidase and a radioimmunoassay technique, respectively. Plasma nonesterified free fatty acids were determined by an enzymatic method. For norepinephrine and epinephrine determinations, blood was collected on lithium heparin with 10 mM sodium metabisulfite and centrifuged for 10 min at 10,000 × g at 0°C; the plasma was stored at −80°C. Catecholamines were selectively isolated from the plasma sample by adsorption on activated alumina, then eluted with 0.1 M perchloric acid. Dihydrobenzylamine was used as internal standard to monitor recovery from the extraction step. Norepinephrine and epinephrine were assayed by high-pressure liquid chromatography using electrochemical (amperometric) detection (Waters HPLC system) as previously described (10,43). All the samples from each individual were analysed at the same time.

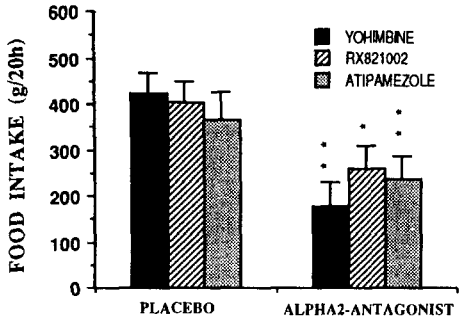

FIG. 2. Comparative effect of acute oral administration of placebo, yohimbine, RX821002 and atipamezole (1 mg/kg) on food intake in 8 dogs. The various alpha$_2$-adrenergic antagonists were administered at 9 a.m., and dogs had access to food at 12 p.m. until 8 a.m. the following day (20-hour period). Values are means ± S.E.M. *$p<0.05$ and **$p<0.02$ when compared to placebo values.

Binding Studies

The platelet membranes were prepared as follows: 20 ml of venous blood was collected over 2 ml of 0.16 M sodium citrate solution and immediately centrifuged at 160 × g for 10 min at room temperature. The platelet-rich plasma (PRP) was collected and the platelet membranes were prepared as previously described (46). The membranes were used for adrenergic receptor determination by a radioligand binding technique, as previously described (44). Total binding of [^{3}H]-yohimbine was determined by incubating 100-μl aliquots of the resuspended membrane preparation [100–200 μg protein assayed by the method of Lowry et al. (31)] with the radioligand in a total volume of 400 μl of binding buffer. Specific binding was defined as the difference between total and nonspecific binding determined in parallel with an excess (10 μM) of phentolamine. The final concentration of radioligand ranged from 0.2 to 15 nM for [^{3}H]-yohimbine. Incubations were carried out for 20 min at 25°C under constant shaking at 100 cycles per min. The samples were filtered through fiberglass filters (Whatman GF/C) placed on a Millipore manifold sampling unit. The filters were washed twice with 10 ml of cold binding buffer, and the radioactivity trapped on the filter was counted in a Packard spectrometer with an efficiency of 35%. The number of sites (B_{max}) and K_d values were calculated with a computer-assisted analysis of binding at saturation and according to Scatchard analysis.

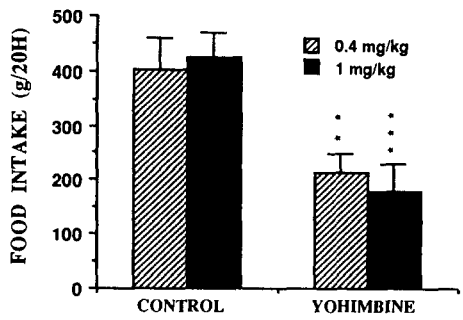

FIG. 3. Comparative effect of acute oral administration of placebo and yohimbine (0.4 and 1 mg/kg) on food intake in 8 dogs. Yohimbine was administered at 9 a.m., and dogs had access to food at 12 p.m. until 8 a.m. the following day (20-hour period). Values are means ± S.E.M. **$p<0.05$ and ***$p<0.02$ when compared to control values.

Chemicals

Yohimbine chlorhydrate® pills, RX801002 [2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline] hydrochloride and atipamezole [4-(2-ethyl-2,3-dihydro-1H-inden-2-yl) 1H-imidazole] hydrochloride were kindly given by Laboratoires Houdé (Paris, France), Reckitt and Colman (Kingston-Upon-Hull, UK) and Farmos-Group Ltd. (Finland), respectively. [^{3}H]Yohimbine (84.5 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Enzymes for nonesterified fatty acid assay (Wako commercial kit) were obtained from Biolyon (Lyon, France) and for glucose (glucose enzymatic color) from Biotrol (Paris, France), and a commercial kit for insulin radioimmunoassay from Institut Pasteur (Paris, France). All other chemicals and organic solvents were of reagent grade.

Data Analysis

Student's paired *t*-test was used for comparisons between matched pairs. The Mann-Whitney test was used for comparisons between the two groups. Differences were considered significant when p was smaller than 0.05.

RESULTS

Alpha$_2$-Antagonists and Food Intake

The effect of equivalent doses (1 mg/kg per os) of yohimbine, RX821002 and atipamezole (or placebo) on food consumption during the three periods was compared as described in the Method section. The results are depicted in Fig. 1. Under placebo, the dogs ate about 50–60% of the total food intake (over 20 h) during the two hours following the access to food (first period). During the 3 successive hours (2nd period), they ate little food (between 5 and 15% of the total food intake), and they consumed the last part (30–40%) during the 13 following hours (3rd period). The three alpha$_2$-antagonists clearly reduced the food intake for the initial 2 hours of food access. In this first period, the reductions were 51, 53 and 64% for yohimbine, RX821002 and atipamezole, respectively (Fig. 1). No significant effect was observed during the 2nd period. Yohimbine (but not RX821002 or atipamezole) significantly reduced food intake during the 3rd period. From the results depicted in Fig. 2, it can be concluded that the three drugs reduced food intake during the 20 h following their acute oral administration and that yohimbine was the most efficient for reducing food intake.

Finally, another experiment was undertaken in order to explore the efficacy of various doses of yohimbine on food intake (0.4 and 1 mg/kg). The anorectic effect of 0.4 mg/kg yohimbine was significant and of the same amplitude when compared to the effect of 1 mg/kg; the reductions of food intake were 31 ± 8 and 47 ± 9% (nonsignificant), respectively (Fig. 3). So for further experiments, the dose of 0.4 mg/kg was used.

The results of this first experiment indicate that the three alpha$_2$-adrenergic antagonists appear to have an anorectic effect in dogs. This anorectic effect is longer lasting with yohimbine, as the reduction of food intake appears at all times after the dogs had access to food over a 20-hour period. So yohimbine was chosen for further experiments to determine the endocrino-metabolic effects of oral alpha$_2$-adrenoceptor antagonists, to explore

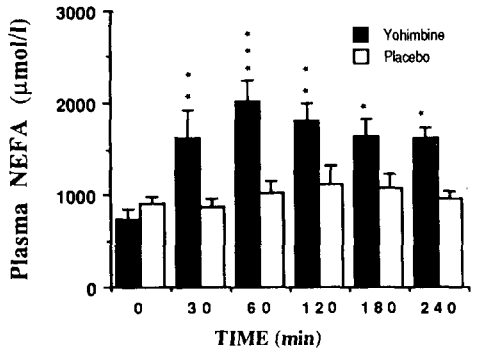

FIG. 4. Time-course effect of acute oral administration of yohimbine or placebo on plasma NEFA levels in 8 dogs. Dogs were fasted overnight (food was suppressed at 5 p.m. the day before). Yohimbine (0.4 mg/kg) or placebo was administered at 9 a.m. Blood samples were collected before ($t=0$ min) and at the various times indicated in the figure. Values are means ± S.E.M. *$p<0.05$; **$p<0.02$ and ***$p<0.01$ when compared to placebo values.

the mechanisms involved in the anorectic effect and to determine if tolerance would develop.

Yohimbine and Endocrino-Metabolic Parameters

The effect of yohimbine (0.4 mg/kg) over the 4 hours following its oral administration (at 9 a.m.) was studied in six dogs after overnight fasting (i.e., food was suppressed at 5 p.m. the day before). The results are depicted in Fig. 4 and Table 1. In these conditions, yohimbine provoked an increase in plasma NEFA levels. The maximal effect of yohimbine was obtained within 60 min, and the lipomobilizing action of the drug persisted over the 4 hours. In contrast, the positive effect on the insulin level did not persist, since 4 hours after yohimbine administration, the plasma insulin concentrations returned to preadministration values (Table 1). The drug induced moderate transient hypoglycemia which was concomitant with the increase of plasma insulin level. Yohimbine also induced a strong increase in plasma norepinephrine and epinephrine (+390 and +160%, respectively), one hour after its administration (Table 1).

Nadolol and Yohimbine Effects

In a previous study (41), we demonstrated that the yohimbine-induced increase in plasma NEFA could be suppressed by pretreatment with a beta-adrenoceptor antagonist (propranolol).

TABLE 1

EFFECT OF ACUTE ORAL YOHIMBINE ADMINISTRATION (0.4 mg/kg) ON PLASMA GLUCOSE, CATECHOLAMINES AND INSULIN LEVELS IN 6 DOGS

Time (min)	Glucose (mmol/l)	Norepinephrine (pg/ml)	Epinephrine (pg/ml)	Insulin (mU/ml)
0	5.2 ± 0.1	404 ± 92	146 ± 22	22.9 ± 6.7
60	4.5 ± 0.2*	2006 ± 505†	384 ± 116*	64.9 ± 23.7†
240	4.7 ± 0.2	1403 ± 335†	256 ± 47	25.0 ± 7.8

Values are mean ± S.E.M.
*$p<0.05$, †$p<0.02$ when compared to values measured at time 0 min.

TABLE 2

EFFECT OF NADOLOL ON THE ACUTE LIPOMOBILIZING ACTION OF YOHIMBINE (EVALUATED BY THE PLASMA NEFA LEVEL, µmol/l) IN 8 DOGS

Time (min)	0	60
Placebo	802 ± 100	2008 ± 226**
Nadolol	237 ± 97†	454 ± 96†

Nadolol (4 mg/kg) or placebo were administered at 5 p.m. and food was suppressed. The following day (9 p.m.), yohimbine (0.4 mg orally) was administered to dogs. Blood samples were collected before ($t=0$ min) and 60 min after yohimbine administration. Values are mean ± S.E.M.
**$p<0.01$ when compared to values measured at $t=0$ min.
†$p<0.02$ when compared to placebo.

The high level of NEFA measured in the plasma after yohimbine administration represents a factor able to modify food intake (Fig. 4). The importance of this factor could be evaluated by treatment with nadolol, a hydrophilic beta-antagonist known to poorly penetrate the central nervous system and to possess a low rate of elimination (elimination half-life: 16–24 hours). This last point was verified by the consequences of a 16-hour pretreatment with nadolol on yohimbine-induced lipomobilization in dogs. Nadolol (4 mg/kg per os) was administered at 5 p.m., and food was suppressed. The following day, at 9 a.m., the animals received yohimbine orally (0.4 mg/kg). Blood samples were taken before and 60 min after yohimbine administration. The results, depicted in Table 2, show that basal plasma NEFA values were lower in treated animals when compared to controls and that the lipomobilizing effect of yohimbine was completely suppressed after nadolol administration.

The consequence of the pretreatment of the dogs with nadolol on the effect of yohimbine on food intake is depicted in Fig. 5. Nadolol (4 mg/kg) was administered the first day (at 9 a.m.) and at 9 p.m. with 1 mg/kg yohimbine the following day. Yohimbine-induced reduction in food intake (measured over the 20-hour period, i.e., from 12 p.m. until 8 a.m. the following day) persisted under beta-blockade.

Chronic Yohimbine Administration on Food Intake and Body Weight

To study the effect of chronic yohimbine treatment on food intake in dogs, the dose of 0.4 mg/kg yohimbine was chosen, since it clearly reduced food intake on acute administration. Spontaneous food intake (without drug) was measured every day in eight dogs over 7 days. After that, they received orally every morning at 9 a.m. 0.4 mg/kg yohimbine and had free access to food from 12 p.m. until 8 a.m. the following day. The treatment persisted for 14 days. During this period, food intake was measured every day. Food intake was also measured during the 14 days following the end of the treatment. Dogs were weighed before treatment (day 7), at the end of the treatment (day 21) and at day 35 (14 days after the end of yohimbine administration). The results of this experiment are reported in Fig. 6 and Table 3. The reduction of food intake provoked by yohimbine persisted over the experimental period. The mean reduction (calculated over the 14-day period) was 40%. The anorectic effect remained significant during the day following yohimbine withdrawal and food intake returned to pretreatment values after 3 days. The treatment provoked a significant weight loss of 4.9%

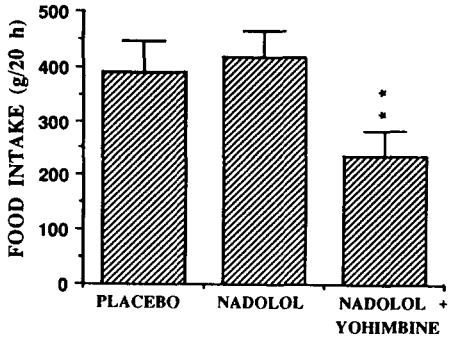

FIG. 5. Effect of acute oral administration of placebo, nadolol (4 mg/kg) or nadolol plus yohimbine (0.4 mg/kg) on food intake in 8 dogs. The first day, the dogs received placebo; the second day, they received nadolol at 9 a.m.; the third day, they received nadolol plus yohimbine at 9 a.m. Food intake was measured over 20 hours for each treatment. Values are mean ± S.E.M. $^{**}p<0.02$ when compared to placebo or nadolol.

when compared to the initial weight. Fourteen days after the end of the treatment, body weight returned to pretreatment values.

Chronic Yohimbine Administration on Alpha$_2$-Adrenoceptors and Endocrino-Metabolic Parameters

Blood samples were taken the day before (at 9 a.m.) the beginning and the day following (at 9 a.m.) the end of yohimbine treatment in order to measure plasma catecholamine, insulin, glucose and NEFA levels. The platelet alpha$_2$-adrenoceptor number ([^{3}H]yohimbine binding sites) was also evaluated. Results are given in Table 4. Neither the number (B_{max}) nor affinity (K_D) of [^{3}H]yohimbine binding sites were modified by yohimbine administration. Plasma norepinephrine and NEFA levels

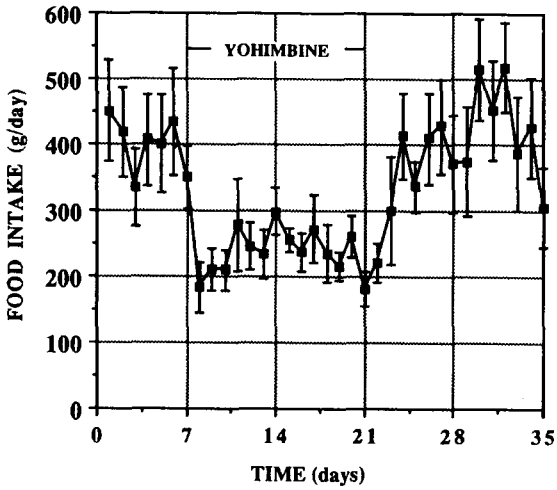

FIG. 6. Effect of chronic administration of oral yohimbine (0.4 mg/day over 14 days) on daily mean food intake of 8 dogs. Spontaneous food intake was measured over a 7-day period. They received yohimbine on day 8 and then every day (at 9 a.m.) until day 21. After then, spontaneous food intake was measured. Food intake was measured each day over 20 hours during the three periods. Values are mean ± S.E.M.

TABLE 3

EFFECT OF 14 DAYS OF ORAL YOHIMBINE (0.4 mg/kg/day) ON FOOD INTAKE AND BODY WEIGHT IN 8 DOGS

Period	Day 7	Day 21	Day 35
Food intake (g/day)	399 ± 15	237 ± 9**	390 ± 21
Body weight (kg)	20.2 ± 2.3	19.2 ± 2**	20.5 ± 2.2

The mean food intake is the mean of daily food intake for each dog during the periods: day 1 to day 7, day 8 to day 21 and day 22 to day 35. Body weight of the dog was evaluated at the end of each period (day 7, 21 and 35). For details, see Fig. 7.

$^{**}p<0.02$ when compared to pretreatment values (day 1 to 7).

were significantly higher, whereas the plasma glucose level was unchanged.

DISCUSSION

The results of the current study indicate that acute oral administration of the various alpha$_2$-antagonist agents tested (yohimbine, RX821002 and atipamezole) reduces food intake in the dog. The basis of this anorectic effect is that yohimbine (and probably the other alpha$_2$-blocking agents used) penetrates the central nervous system and blocks central alpha$_2$-adrenoceptors and, as a consequence, stimulates the noradrenergic central nervous system and sympathetic nervous system.

Evidence for direct activation of the sympathetic nervous system after alpha$_2$-adrenergic antagonist administration was the increased level of plasma catecholamines after yohimbine administration in dogs [(43,45) and present study] and humans (11, 18, 36). The effect on the noradrenergic central nervous system was also directly demonstrated by Peskind et al. (36) in humans; oral yohimbine (0.65 mg/kg orally) increased the norepinephrine concentration in the cerebrospinal fluid. Indirect demonstration was that yohimbine causes arousal in humans and behavioural excitation in other species (14). However, Lang and Gershon (19) demonstrated differences in the behavioural and cardiovascular effects of yohimbine in several species and concluded that the dog seems to be the animal of choice to compare effects with those seen in humans.

TABLE 4

EFFECT OF CHRONIC ADMINISTRATION OF ORAL YOHIMBINE (0.4 mg/kg/day OVER 14 DAYS) ON PLATELET [^{3}H]YOHIMBINE BINDING SITES (B_{max} AND K_D), PLASMA NOREPINEPHRINE, NEFA AND GLUCOSE LEVELS IN 8 DOGS

	Before Treatment	14 Day Treatment
B_{max} (fmol/mg protein)	222 ± 14	197 ± 7
K_D (nmol/l)	1.3 ± 0.2	1.3 ± 0.05
Norepinephrine (pg/ml)	589 ± 205	844 ± 189*
NEFA (μmol/l)	579 ± 41	946 ± 92*
Glucose (mmol/l)	4.08 ± 0.8	4.03 ± 0.6

For binding analyses, platelet membranes were incubated for 30 min at 25°C with different concentrations of [^{3}H]yohimbine as described in the Method section and total binding sites were determined by Scatchard analysis. Blood was collected the day before treatment (9 a.m.) and the day following the last administration of yohimbine (9 a.m.). For details, see Fig. 7.

$^{*}p<0.05$ as compared to corresponding values.

The three alpha$_2$-adrenergic antagonists used in this study provoked a reduction of food intake over the 20 hours following their administration (Fig. 2). However, the monitoring of food intake over successive periods (Fig. 1) after the dogs had access to food revealed that, whereas atipamezole, RX821002 and yohimbine reduced food ingestion during the first period (12 p.m. to 2 p.m.), yohimbine only reduced food intake during the third period (5 p.m.–8 a.m. the following day). The lack of data concerning the pharmacokinetics and the oral bioavailability for each compound in the dog does not permit a full discussion of this point. The pharmacokinetics of yohimbine have only recently been examined in humans (35). The drug was rapidly absorbed and rapidly eliminated from the plasma (elimination half-life 0.6 ± 0.26 hour). These data do not fit with certain pharmacodynamic effects of yohimbine: yohimbine per os increased plasma NEFA and catecholamine levels over a 3–6-hour period in humans [(34) and personal results] and in dogs [(43,45) and present data]. Moreover, Goldberg et al. (15) reported some long-lasting effects of yohimbine (irritability, fatigability), and Berlin et al. (4) described 24-hour effects of yohimbine on the velocity of adrenaline-induced platelet aggregation in humans. Accordingly, with the hypothesis of Owen et al. (35), one can suggest that yohimbine is eliminated primarily through metabolism, since virtually no drug was eliminated in the urine. Perhaps the effect of yohimbine on food intake in the dog resides in the appearance of an active metabolite.

An anorectic effect of high doses of yohimbine or rauwolscine (5 mg/kg intraperitoneally) has been previously described in mice (7). Lean mice are much less sensitive to the effect of the drug, whereas the genetically obese (ob/ob) mutant is more sensitive (a significant effect appears with 3 mg/kg yohimbine). The authors suggested that modifications of norepinephrine release by manipulation of alpha$_2$-adrenergic receptors can alter food intake. From a theoretical point of view, it is interesting to remark that central (16) or systemic (39) administration of clonidine (alpha$_2$-adrenoceptor agonist) induces hyperphagia in the normal rat. The orectic effect of clonidine has also been described in the dog (23) and in the monkey, where it induced a significant weight gain after a 3-day treatment (41). This effect on food intake was suppressed by yohimbine but not by prazosin pretreatment, suggesting a specific effect on alpha$_2$-adrenoceptors. Moreover, yohimbine alone (5 mg/kg intramuscularly) significantly reduced food intake in the monkey (41). Thus, taking in account the possible existence of a tonic alpha$_2$-adrenergic inhibition of the satiety center located in the paraventricular nucleus, alpha$_2$-antagonist compounds are putative anorectic agents. Finally, the effect of alpha$_2$-antagonists seems to be specific to feeding. Using the oral route and 0.33 mg/kg, Montastruc et al. (33) demonstrated that yohimbine induced an antidiuretic response without modifying the daily water intake in the dog.

In the present study, the anorectic effect of alpha$_2$-adrenoceptor antagonists was obtained in dogs with low oral doses (1 mg/kg), whatever the chemical structure of the alpha$_2$-antagonist tested. Moreover, a significant inhibitory effect on feeding was obtained with 0.4 mg/kg oral yohimbine. This dose of yohimbine provoked an increase in the plasma norepinephrine level and, to a lesser extent, in the plasma epinephrine level. Surprisingly, the increase in norepinephrine, NEFA and insulin plasma levels in dogs was of the same extent as that found in a previous study in which 0.5 mg/kg yohimbine was administered intravenously as a bolus (43). It can be concluded that there is good yohimbine bioavailability after oral administration in the dog and that this animal model is very sensitive to alpha$_2$-antagonists, as previously suggested by Lang and Gershon (19) and Lang et al. (20).

We previously demonstrated that the increase in NEFA plasma level observed after yohimbine administration is linked to a beta adrenergic stimulation of the lipolytic pathways in the adipose tissue, as a beta blocker completely suppressed the observed responses (43). The increased plasma NEFA level observed after yohimbine administration is a factor that can reduce food intake ("lipostatic theory"), since the hypothalamus area integrates the metabolic status of the body and could modulate ingestive behavior. All hormones or pharmacological substances inducing a short-term lipid mobilization are able to reduce appetite. In our experiments, pretreatment with nadolol (a peripheral hydrophilic beta-adrenoceptor antagonist chosen for its long half-life and for its low capacity to penetrate into the central nervous system) entirely suppresses the yohimbine-induced lipomobilization (Fig. 5) but does not modify the anorectic effect of yohimbine. So the participation of an increased NEFA plasma level in the anorectic effect of yohimbine can probably be ruled out.

The mechanism of anorectic action of alpha$_2$-adrenoceptor antagonists is rather complex. These drugs can directly block the alpha$_2$-adreceptors located on the medial hypothalamus, particularly the nucleus paraventricularis, the activation of which stimulates feeding (16,26). This is sustained by the hypothesis that alpha$_2$-stimulation of the nucleus paraventricularis is tonic in nature as in the central nervous noradrenergic system (36).

On the other hand, the lateral hypothalamus and the perifornical area contain beta-adrenergic receptors, the activation of which reduce food intake (27). The reduction of food intake caused by epinephrine (28) or salbutamol (2) injection in the perifornical area was more efficiently counteracted with beta$_2$- than with beta$_1$-adrenergic antagonists. These results suggest that the receptors involved are mainly of beta$_2$ subtype. Moreover, the anorectic effect of a releaser of norepinephrine, diethylpropion, is also prevented by preinjection of propranolol in the perifornical area (2). So the anorectic action of yohimbine could also be indirectly brought about by norepinephrine stimulation of lateral hypothalamus beta$_2$-adrenoceptors.

The effect of yohimbine could also be related to an interaction with 5-HT receptors located in the paraventricular nucleus. The stimulation of the 5-HT receptor of this area reduces food intake, whatever the origin of hyperphagia (stressful stimuli, muscinol injection or drug-induced glucoprivation) (6, 8, 38). Recently, Convents et al. (9) demonstrated that [^{3}H]rauwolscine (the isomeric form of yohimbine) binds with high affinity to 5-HT1-like receptors in human brain cortex membranes. So an interaction of yohimbine with 5-HT receptors located in the paraventricular nucleus cannot be excluded. However, it has been demonstrated that serotonin exerts its anorectic effects through an action on 5-HT$_{1B}$ receptors (32). In fact, rauwolscine binding closely agrees with serotoninergic compounds active on 5-HT$_{1A}$ but not with those active on 5-HT$_{1B}$, 5-HT$_{1C}$ or 5-HT$_{1D}$ (9). Moreover, the various alpha$_2$-antagonists used in the present experiment were active on food intake, whereas they structurally differ from yohimbine.

Apart from the theoretical implications of this study, these results may be of clinical interest. The appetite-suppressing effect of yohimbine (and other alpha$_2$-adrenergic antagonists) might be of benefit in the treatment of eating disorders and of overweight. However, a pharmacological aid in slimming therapy is generally proposed over a long period. So we tested the effect of chronic yohimbine administration (14 days) on food intake and on certain endocrino-metabolic indexes in dog. In order to check that the treatment did not modify alpha$_2$-adrenoceptors, we also measured the [^{3}H]yohimbine platelet binding sites before and after the end of the treatment. The anorectic effect of yohimbine (0.4 mg/kg/day) persisted over the experimental period (Fig. 6 and Table 3). The animals lost body weight (about 5% of their initial weight). The effect of yohimbine on food in-

take was not accompanied with other major observable behavioural modifications. No modification of platelet [^{3}H]yohimbine binding sites was observed indicating no alteration (at least at the peripheral level) in alpha$_2$-adrenoceptor number (Table 4). Finally, the persistence of an action of yohimbine during treatment can be assumed from the higher plasma NEFA and norepinephrine levels measured the day following the end of the treatment period when compared to pretreatment values (Table 4).

Two attempts to include yohimbine in a weight-reducing regime have been made in humans. Yohimbine administered for 8 weeks through a clinical trial performed on two parallel groups was without any impact on weight loss (3). Zahorska-Markiewicz et al. (49) demonstrated, in a cross-over study, that yohimbine application (15 mg/day for 7 weeks) increased plasma NEFA levels and weight loss and had an impact on exercise-induced energy expenditure without other side effects. These investigations led to conflicting results. Further experiments are necessary to assess the putative interest of yohimbine (and other new alpha$_2$-adrenergic antagonists) in obesity therapy. The lipolytic action of alpha$_2$-adrenergic antagonists demonstrated in the dog [(43,45) and present study] and in humans (11,49) is now well documented. Moreover, the results presented here demonstrate the anorectic effect of alpha$_2$-adrenergic antagonists in the dog. An effect of these compounds on appetite reduction (or satiety increase) remains to be evaluated in humans.

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