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The Role of Opiate Mechanisms in the Development of Tolerance to the Anorectic Effects of Amphetamines[1]

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NENCINI, P. *The role of opiate mechanisms in the development of tolerance to the anorectic effects of amphetamines.* PHARMACOL BIOCHEM BEHAV **30**(3) 755–764, 1988.—To study the role played by opiate mechanisms in the tolerance to the anorectic effects of amphetamines, the influence of chronic treatment with d,l-amphetamine (AMPH) on the effects of the selective kappa opiate agonist U50488H (U50), of morphine (MORPH) and of diazepam (DZP) on food and water intake was evaluated in rats. Since diuresis is selectively enhanced by kappa agonists, its sensitivity to chronic AMPH was also evaluated. On the first day of AMPH treatment the feeding response to U50 was depressed. On day 9, when tolerance to the anorectic effects of AMPH had developed, this response was enhanced and prolonged. U50-mediated diuresis was not increased in the AMPH group. AMPH however produced diuresis by itself and this effect may be responsible for the increased water intake that developed during chronic treatment. The administration of MORPH (on day 17), but not of DZP (on day 13), produced a similar pattern of response. Interruption of AMPH treatment brought about a slow normalization of response to U50, that appeared to be completed after 17 days. An increase in feeding response to U50 was also obtained after 14 days of cathinone administration, confirming the amphetamine-like properties of this drug. In order to evaluate the possibility that preventing sensitization of opiate mechanisms could also prevent tolerance to anorectic effects of AMPH, we chronically administered MORPH in combination with AMPH, obtaining a further reduction of feeding and an apparent slowing in tolerance development. However, such a reduction was also obtained acutely, although MORPH alone produced feeding stimulation. We suggest that opiates may both activate and inhibit feeding and that AMPH inhibits the activatory branch and works synergically with the inhibitory branch. The prolonged inhibition of the activatory branch causes its compensatory hypertrophy resulting in hypersensitivity to exogenous opiates.

Amphetamine Cathinone U50488H Morphine Feeding Drinking Diuresis Tolerance

BOTH central and peripheral effects of amphetamines are considered the result of activation of catecholaminergic mechanisms [21]. Since in several areas of CNS both noradrenergic and dopaminergic circuits are closely interconnected with the activity of endogenous opiates [2], the problem of whether these interactions take part in the amphetamine effects has been dealt with in several studies. Thus, evidence has been provided that, in producing the analgesic effects of amphetamines, opiate and monoaminergic mechanisms cooperate. This cooperation results in the possibility both of obtaining a supra-additive analgesia by administering amphetamines and opiates in combination [7, 30] and of reversing the inhibition of nociception produced by amphetamines with the opiate antagonist naloxone [15,25].

Inhibition of ingestive behavior is another amphetamine effect in which an interaction between catecholaminergic and opiatergic mechanisms may play a role. The seminal observation by Holtzman that naloxone produces anorexia [12] led to the finding that opiate mechanisms participate in the central regulation of feeding behavior. So far, stimulation of food intake has been produced by activation of opiate receptors in the hypothalamus, nucleus accumbens and ventral tegmental area [9, 19, 24]. At least in the hypothalamus catecholamines seem to play a role in this opiate-mediated stimulation, since beta-endorphin has been found to activate an alpha-noradrenergic pathway which inhibits the activity of satiety neurons [20]. The opposite possibility that monoaminergic mechanisms inhibiting ingestive behavior modulate the activity of opiatergic circuits has received less attention. Nevertheless, there is evidence that in guinea pigs chronic amphetamine administration increases the hypothalamic content of immunoreactive beta-endorphin and also enhances sensitivity to the anorectic effect of naloxone [27]. Since the activation of hypothalamic dopaminergic and/or beta-noradrenergic mechanisms is considered crucial for the expression of amphetamine anorectic effects [18], these changes in the opiate system are probably secondary to a prolonged catecholaminergic overactivation. Likewise, in rats, chronic administration of the serotonergic anorectic

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fenfluramine produces accumulation of hypothalamic endorphins, probably decreasing their utilization [10]. An increase in brain enkephalin levels, as well as up-regulation of opiate receptors and sensitization to the actions of exogenous opiates, is caused by conditions of reduced interaction between endogenous opiates and their receptors [31,32]. If these mechanisms are at work during chronic treatment with anorectic drugs, we would expect an increased sensitivity to the opiate effects, including feeding stimulation. In a previous paper we have evaluated the possibility that such a sensitization develops during chronic treatment with cathinone (CATH) [26], an anorectic and amphetamine-like compound derived from khat [13]. We found that the apparent normalization of food intake obtained after 9 days of daily administration of CATH was associated with a remarkable increase in the feeding response to the administration of U50488H (U50), a selective ligand of kappa-opiate receptors [34].

In the present study we have further analyzed the presence of a functional sensitization to opiates during chronic administration of amphetamine-like anorectics. We wanted to know (1) if sensitization to the opiate-mediated feeding caused by chronic CATH administration was an amphetamine- like effect; (2) if sensitization was restricted to kappa-opiate mechanisms and was reversible; (3) if sensitization took part in the development of tolerance to the anorectic effects of amphetamine-like agents.

We therefore studied the effect of daily administration of d,l-amphetamine (AMPH) on U50-, morphine (MORPH)-, or diazepam (DZP)-stimulated food intake in a free-feeding paradigm. For the sake of comparison, we repeated this experiment substituting d,l-cathinone (CATH) for AMPH, but restricting the interaction to U50. The reversibility of sensitization was studied by comparing the feeding response to U50 before, during and after chronic AMPH treatment. Finally, assuming that chronic stimulation of opiate mechanisms could prevent their sensitization produced by AMPH, we chronically administered MORPH in combination with AMPH and evaluated the influence of this drug combination on the development of tolerance to the anorectic effects of AMPH.

Besides food intake, diuresis and water intake were also studied, the first for its selective sensitivity to kappa-mediated stimulation [17], the second for its physiological relationship with both food intake and urine output.

GENERAL METHOD

Animals

The subjects were 72 Sprague-Dawley male rats (Morini, San Polo D'Enza, RE, Italy) with an average weight of 391.9±4.6 g (mean±SEM) at the beginning of the study. Four weeks after their arrival at the laboratory colony, they were housed singly in metabolic cages (Tecniplast Gazzada) at 21°C with a daylight cycle (the length of the light phase varied between 10 and 12 hr and the onset between 6 and 7:30 a.m.). The animals had free access to food and water. Food was made available as a gross powder, by grinding chows (Standard Diet 4RF21, Charles River) immediately before presentation. We avoided dispensing chows to minimize spillage due to behaviors not connected to food intake (i.e., stereotyped gnawing). During the first week, the rats were allowed to adapt to the new environment by restricting manipulation to a daily handling for weight record.

Independent Measures

During the experimental procedures described below, food and water intake and urine output were measured by weighing (approximately 0.1 g) food receptacles, water bottles and urine cylinders before and 2 and 5 hr after drug administration, as described previously [26]. To prevent evaporation, urine cylinders contained a layer of mineral oil.

Data Analysis

The data were represented as mean±SEM. When the experimental design compared 2 groups only, differences were evaluated using the two-tailed Student's *t*-test. In the other cases data were analyzed using analysis of variance (ANOVA) and, subsequently, group comparisons were performed using Duncan's multiple-range tests. Correlations were calculated by regression analysis.

Drugs

AMPH monobasic racemic (BDH) was dissolved in diluted chloridric acid and the solution brought to neutrality by adding diluted alkali. U50488H (trans-±-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cycloexyl]-benzene-acetamide methane sulfonate; Upjohn Company), racemic CATH (alpha-aminopropiophenone hydrochloride; UNFDAC), and MORPH hydrochloride (Carlo Erba) were freshly dissolved in distilled water to a final volume of 1 ml/kg. DZP was given as injectable Valium (Hoffman-LaRoche).

EXPERIMENT 1

Procedure

The experiment was performed according to a 2×2 design in which 2 groups of rats were chronically treated with water or AMPH. Each group was then subdivided into 2 groups and tested for the effects produced by U50, or MORPH, or DZP on food intake, water intake and urine output. In detail, rats were assigned to 2 different groups receiving daily intraperitoneal (IP) injection of water or 4 mg/kg AMPH, respectively, for 19 days. On day 1, immediately after the 5 hr measures of food and water intake and of urine output, both AMPH and water groups were divided into two groups receiving water or U50 (8 mg/kg, IP), respectively. In a previous study the U50 dose adopted has been found to be fully active in stimulating food intake without having noticeable sedative effects [26]. Food and water intake and urine output were measured at 2 and 5 hr after water or U50 administration (i.e., 7 and 10 hr after the previous injection of water or AMPH). The same experiment was repeated on day 9 when there were no differences between water and AMPH groups for cumulative 5 hr food intake; on day 13, when 4 mg/kg DZP substituted for U50; and on day 17, when 2 mg/kg MORPH substituted for U50. Rats administered with MORPH and DZP were the same that had received U50.

During the experiment we observed a progressive increase in both food and water intake in the AMPH group (Fig. 1 and 2); we therefore tested the possibility that the polydipsic response to chronic AMPH administration was secondary to the increase in food intake. On day 18, after water or AMPH administration, 6 rats of each of the 2 groups were food deprived for the 5 hr and water consumption was measured at 2 and 5 hr.

Since we obtained evidence that chronic AMPH treat-

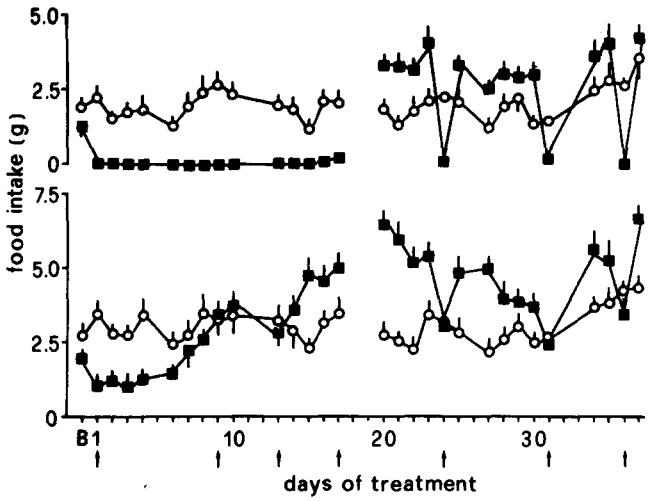

FIG. 1. The effects of chronic IP administration of water (open circles) or AMPH 4 mg/kg (closed squares) on food intake at 2 hr (upper panel) and 5 hr (lower panel). Both groups received IP water from day 20 on (post-tolerance follow-up in the AMPH group). Arrow indicate days in which 5 hr after water or AMPH rats received water or U50 (days 1, 9, 24, 31 and 36), or DZP (day 13), or MORPH (day 17). Note that on days 24, 31 and 36 (post-tolerance period) AMPH group received again 4 mg/kg AMPH. Each point is the mean (±SEM) of 12 rats. Marks for significant differences (determined by two-tailed Student's *t*-test) are omitted for the sake of clarity. B: baseline values.

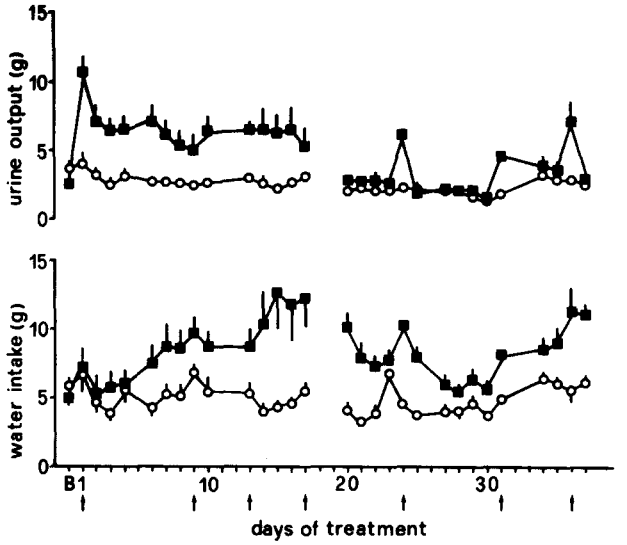

FIG. 2. The effects of chronic IP administration of water (open circles) or AMPH 4 mg/kg (closed squares) on 5 hr cumulative urine output (upper panel) and water intake (lower panel). Same experiment and same symbols shown in Fig. 1.

ment produced sensitization to opiate-mediated hyperphagia, we studied the reversibility of such a sensitization by switching the AMPH group to water treatment from day 20 to day 38 (the water group continued to receive water). On days 24, 31 and 36 the AMPH group again received AMPH treatment (4 mg/kg) and the experiment performed on day 1 was repeated, half of each group receiving U50 5 hr after water or AMPH injection. The rats that received U50 were the same administered with U50, MORPH and DZP in the earlier trials. To further check the specificity of the AMPH effect on opiate-produced hyperphagia, a cross-over experiment was also performed on day 38. The water group received AMPH (4 mg/kg, IP) whereas the AMPH group continued to receive water. The experiment performed on day 1 was then repeated.

RESULTS

Effects of Chronic AMPH Administration

Figure 1 shows the pattern of food intake during and after a period of chronic AMPH administration (4 mg/kg, IP). In the first 2 hr after AMPH injection food intake was completely suppressed and rats showed a remarkable stereotyped behavior consisting mainly of head twisting. The behavior, as well as suppression of food intake, was maintained during the entire period of AMPH treatment (i.e., 19 days). Cumulative food intake at 5 hr was also reduced by AMPH, but this effect was statistically significant only during the first 4 days of treatment. From day 5 to 14 food intake at 5 hr was the same in both AMPH and control groups; from day 15 it was statistically higher in the AMPH-treated animals. The anorectic effect of AMPH was associated with a reduction of body weight, which reached the nadir on day 8 of treatment (−4.1% and +1.2% compared with baseline values in the AMPH- and water-treated groups, respectively) and was significant with respect to controls till day 15 of treatment.

Substitution of water for AMPH produced a remarkable overshoot of food intake at both 2 and 5 hr, which slowly faded during the period of post-AMPH observation. Body weight progressively increased and at the end of the study both AMPH- and water-treated groups showed similar increments compared with baseline values (3.9% and 5.5%, respectively). Administration of AMPH (4 mg/kg) on days 24, 31 and 36 (days 5, 12 and 17 of the post-AMPH period) produced both stereotypes and suppression of food intake at 2 hr. At 5 hr only the overshoot in food intake was prevented by AMPH and both AMPH and water-injected groups ingested the same quantity of food.

Both cumulative water intake and urine ouput at 5 hr are shown in Fig. 2. On the first day of treatment, AMPH produced a striking diuretic effect, which was almost complete at 2 hr (data not shown). On the second day the diuretic response to AMPH was halved and then stabilized to a level that was roughly double the control urine output. Water intake during the first 2 hr was very low in both water and AMPH groups and there were no significant differences between the two groups (data not shown). Acute AMPH administration failed to reduce cumulative water intake at 5 hr and, given chronically, produced a progressive increase of drinking so that by day 15 water intake was roughly 3 times the control values. The interruption of AMPH administration was followed by a complete normalization of urine output, but did not produce an overshoot of drinking, as in the case of food consumption. Thus, water intake was always lower than the level reached in the last days of AMPH treatment, even though higher than control levels. In spite of this apparent normalization of water intake, AMPH administration on days 24, 31, and 36 (post-AMPH period) produced a significant increase in drinking (along with an increase in urine output), as in the last period of chronic AMPH treatment.

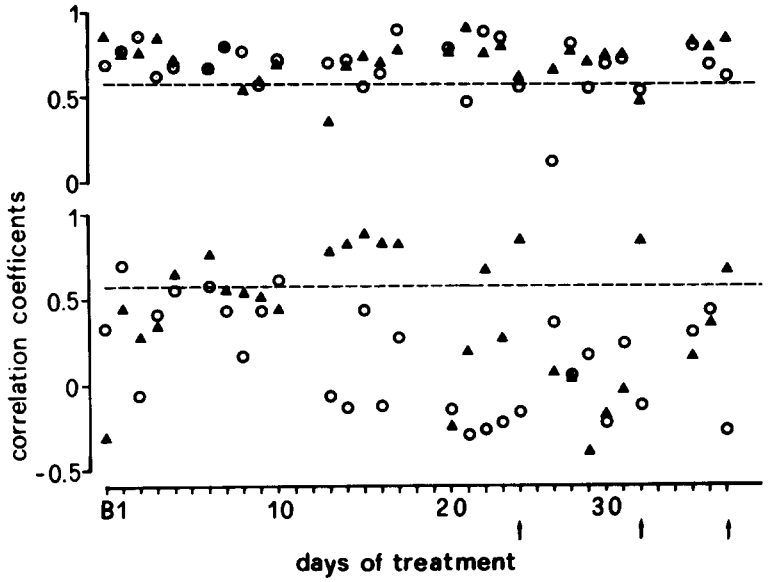

FIG. 3. Coefficients of correlation between cumulative 5 hr water and food intakes (upper panel) or water intake and urine output (lower panel) in the water- (open circles) and AMPH- (closed triangles) treated groups. Dotted lines indicate the level of significant correlation ($p<0.05$) and arrows administration of AMPH during the post-tolerance period.

TABLE 1
WATER INTAKE IN 5 HR FOOD DEPRIVED RATS

	Water Group		AMPH Group	
Food	Yes	No	Yes	No
0–2 hr	3.37± 0.65	2.15± 0.35	1.78± 0.14	1.62± 0.1
0–5 hr	4.80± 0.53	2.95± 0.36	10.53± 2.13	12.0± 4.75

On day 18, immediately after water or AMPH administration (4 mg/kg, IP), half of each group (i.e., 6 rats for each group) were food deprived for the 5 hr of post-injection observation. Values are expressed as mean g (±SEM). A two-way ANOVA shows significant effects for treatment only, $F(1,20)=7.9$, $p<0.05$, at 5 hr.

The dependence of water intake on food consumption was tested. The analysis of correlations shows that water and food intakes at 5 hr were usually statistically correlated throughout the experiment in both water and AMPH-treated groups (Fig. 3). In contrast, water intake did not correlate with urine output in the controls. However, in the AMPH-treated group these two variables became correlated from day 13 on. On the interruption of AMPH administration correlation ceased, but each time AMPH was given again (i.e., on days 24, 31 and 36) correlation returned. To further determine whether the polydipsia observed in AMPH-treated rats depended on the enhanced food intake, on day 18 of treatment, half of each of the two treatment groups were food deprived for the 5 hr of post-injection observation. The results show that water intake was significantly increased in the AMPH-treated animals, whether they received food or not (Table 1).

Effects of Chronic AMPH on Drug-Mediated Stimulation of Feedng

The influence of AMPH treatment on food intake stimulated by U50 was studied on days 1, 9, 24, 31 and 36 from the beginning of AMPH administration. Figure 4 shows the values of food intake at 2 and 5 hr after U50 administration (i.e., 7 and 10 hr after the last AMPH injection, respectively). In water-pretreated rats, U50 administration invariably produced a significant increase in food intake at 2 hr. This effect was short-lasting and at 5 hr there were no differences in food intake between the U50- and water-injected groups. Feeding response to U50 in the AMPH-treated group depended on the day of AMPH treatment. On day 1, food intake was still deeply depressed in AMPH-pretreated animals at both 7 and 10 hr and U50 only slightly counteracted this anorectic effect. On day 9, when there were no differences between AMPH- and water-pretreated animals for 5 hr food intake, both 2 and 5 hr after administration U50 produced much more food ingestion in the AMPH than in the control group. Differences in the feeding response to U50 were particularly noticeable at 5 hr, when there was a significant interaction between AMPH and U50 treatments [two-way ANOVA, $F(1,20)=6.3$, $p<0.05$].

Discontinuation of AMPH treatment produced a slow normalization of the feeding response to U50. This response on day 24 (7 days from the interruption of AMPH) was indeed close to that obtained on day 9, as at 5 hr we again observed a significant interaction between AMPH and U50 treatments, $F(1,20)=6.2$, $p<0.05$. On day 31 the interaction

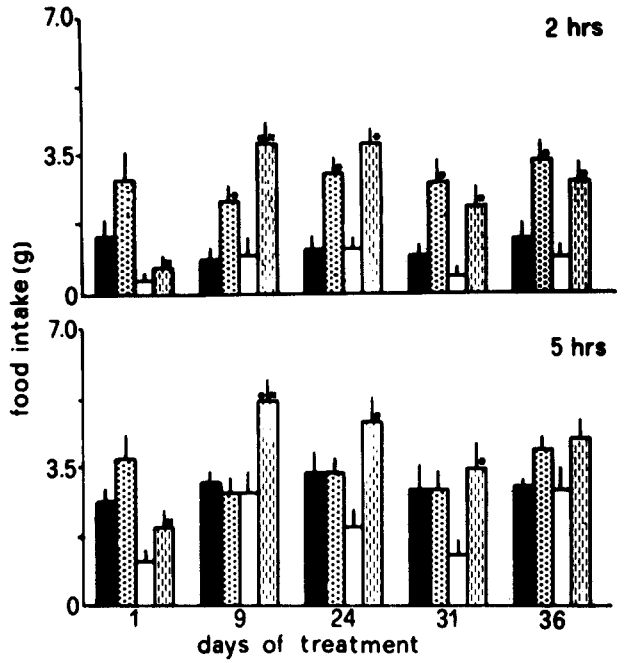

FIG. 4. Effects of water or U50 (8 mg/kg) given IP 5 hr after water or AMPH 4 mg/kg on 2 hr (upper panel) and 5 hr (lower panel) food intake. Closed and dotted bars represent chronic water administered groups treated with water or U50, respectively. Open and striped bars represent chronic AMPH-administered groups treated with water or U50, respectively. Each bar represents the mean (±SEM) of 6 animals. *$p<0.05$ vs. respective water-treated group, ×$p<0.05$ vs. water-pretreated group (Duncan's test).

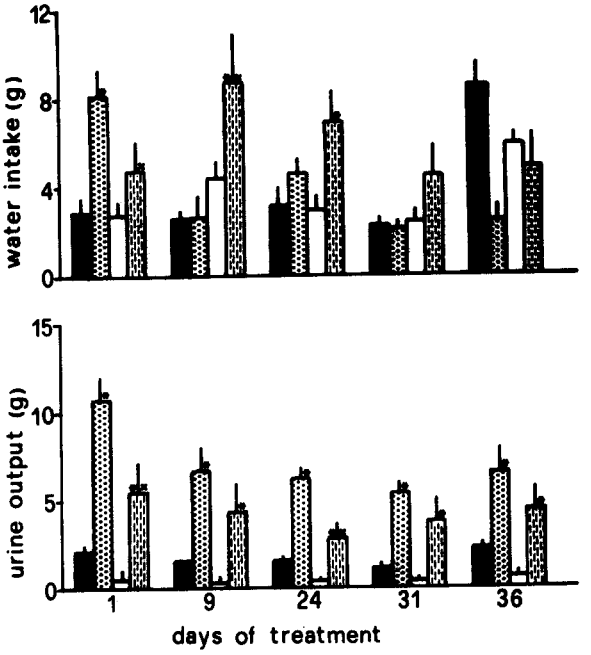

FIG. 5. Effects of water or U50 (8 mg/kg) given IP 5 hr after water or AMPH 4 mg/kg on 5 hr water intake (upper panel) and urine output (lower panel). Same experiment and same symbols as in Fig. 4.

was no longer significant, although in AMPH-pretreated rats U50 still significantly increased food intake at 5 hr. Complete normalization in the response to U50 was reached on day 36 (17 days after the interruption of AMPH treatment) and stimulation of food intake was detectable in both water- and AMPH-pretreated groups at 2, but not at 5 hr. A similar pattern of response was observed when water intake was considered (Fig. 5).

Since diuresis is a typical response to kappa-opiate agents, this effect was studied in evaluating the specificity of the enhancement of feeding response to U50 observed in the AMPH-pretreated rats. As Fig. 5 shows, diuresis produced by U50 was never enhanced in these animals, but in some circumstances (i.e., days 1 and 24) it was significantly reduced.

Table 2 shows the results of a crossover experiment performed on day 38, in which the water-treated group received AMPH (4 mg/kg) for the first time. As expected, food intake was significantly depressed at both 2 and 5 hr, and feeding response to U50 was prevented. In the post-AMPH group that received water, feeding response to U50 was that typically observed in the control animals, i.e., it was increased at 2 but not at 5 hr.

The effect of chronic AMPH treatment on the food and water intakes produced by both MORPH and DZP was also evaluated and the results are shown in Fig. 6. The response to MORPH was similar to that observed in U50. On day 17, the administration of MORPH (2 mg/kg, IP) produced an increase of food intake that at 2 but not at 5 hr was significantly higher in the AMPH-pretreated rats. As in the case of U50, a significant interaction between AMPH and MORPH treatment was noticed at 5 hr, $F(1,20)=14.9$, $p<0.01$. Water intake response paralleled that of food intake. DZP (4 mg/kg) produced a small increase of food intake at 2 hr in both water- and AMPH-pretreated rats [two-way ANOVA, $F(1,20)=5.8$, $p<0.05$, for DZP treatment]. At 5 hr feeding response to DZP was higher in the AMPH-pretreated animals. However AMPH pretreatment increased 5-hr food intake also in the water-treated group, $F(1,20)=8.0$, $p<0.05$, for AMPH pretreatment). Thus, the effect of AMPH ap-

TABLE 2

FOOD INTAKE IN THE CROSSOVER EXPERIMENT

	Water Group		AMPH Group	
Pretreatment	AMPH 4 mg/kg		Water	
0–2 hr	0.17 ± 0.17		3.04 ± 0.43	
0–5 hr	1.25 ± 0.34		5.18 ± 0.48	
Treatment	Water	U50	Water	U50
0–2 hr	0.4± 0.15	1.6±† 0.45	1.93± 0.81	4.1±* 0.64
0–5 hr	2.23± 0.39	2.58± 0.39	4.38± 0.71	4.68± 0.74

On day 38 controls (i.e., water group) were injected with AMPH and the AMPH group continued to receive water. After 5 hr both groups were split into two groups receiving water or U50 (8 mg/kg, IP), respectively. Values are expressed as mean g (±SEM). *$p<0.05$ vs. the respective water group and †$p<0.05$ vs. AMPH group (Duncan's test).

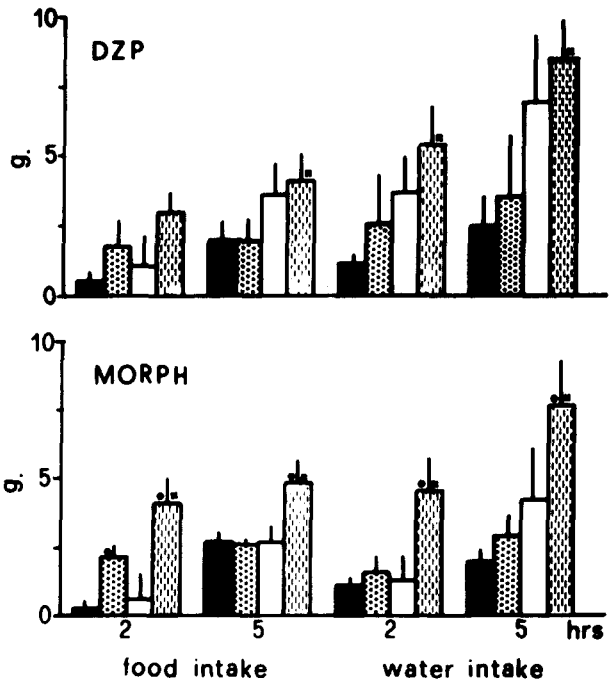

FIG. 6. The effects of water or DZP (4 mg/kg, upper panel) or MORPH (2 mg/kg, lower panel) given IP 5 hr after water or AMPH 4 mg/kg on food and water intake. DZP and MORPH were administered on day 13 and 17, respectively, of chronic AMPH administration. Same symbols as Figs. 4 and 5, with the difference that DZP or MORPH substitute for U50.

peared to be a general increase in food intake rather than an effect on the response to DZP. A closely similar pattern of response was obtained with water intake.

EXPERIMENT 2

Procedure

In a previous study, adopting a rather different protocol from that described in Experiment 1, we observed that chronic administration of CATH sensitized rats to stimulation of food ingestion produced by U50. To compare the effects of AMPH and CATH, we repeated Experiment 1 substituting CATH for AMPH. In detail, 24 rats were assigned to 2 groups receiving CATH (8 mg/kg, IP) or water, respectively. After 14 days of treatment rats received water or U50, according to the procedure adopted on day 1 of the previous experiment. Food and water intake and urine output were measured as described.

RESULTS

U50 was given on day 14 of chronic CATH administration when tolerance to the anorectic effects of CATH had developed (food intake at 5 hr was 4.32±0.57 and 3.82±0.87 g in the water- and CATH-treated group, respectively). As expected, U50 produced a response pattern on food intake and on diuresis close to that caused in chronically AMPH-treated animals (Fig. 7). Food intake at 5 hr was strongly enhanced, whereas diuresis was slightly reduced. Both CATH-pretreated groups showed an increase in water intake, that was not further enhanced by U50 administration.

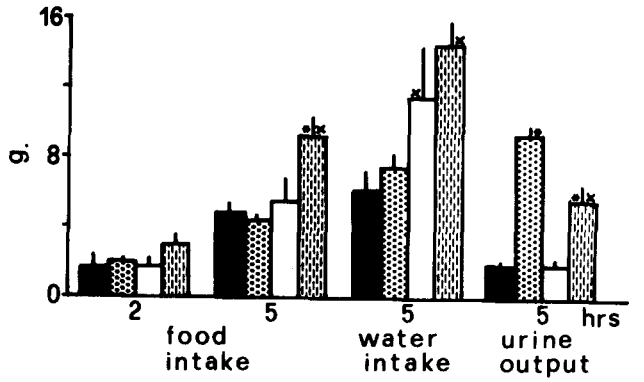

FIG. 7. The effects of water or U50 (8 mg/kg) given IP 5 hr after the last of 14 daily administrations of CATH (8 mg/kg) on food and water intakes and urine output. Same symbols as in Fig. 4 with the difference that CATH substitutes for AMPH.

EXPERIMENT 3

Procedure

The fact that chronic AMPH administration enhanced opiate-mediated stimulation of feeding prompted us to find out whether sensitization of opiate mechanisms was involved in the tolerance to the anorectic effects of AMPH. If this sensitization were due to a persistent inhibition of opiate mechanisms caused by AMPH, we argued that maintaining the opiate tone could slow the development of sensitization of opiate mechanisms and hence slow tolerance to AMPH anorectic effects. We tested this hypothesis by giving MORPH in combination with AMPH. MORPH was preferred to U50 to ensure that the cumulative diuretic effects of both AMPH and U50 did not cause a hydric unbalance that might affect ingestive behavior.

Twenty-four rats were assigned to 4 groups. The animals received subcutaneously (SC) water or MORPH 1 mg/kg and, 10 min after, water or AMPH (4 mg/kg, IP), according to a 2×2 design in which all treatment combinations were represented. Since MORPH effects have a very rapid onset also when the drug is administered SC, a latency of 10 min between the two treatments was considered appropriate to ensure that opiate mechanisms activated before the supposed inhibitory effect of AMPH was operating. Treatment lasted 10 days and independent measures were taken as described except that total daily food intake was also recorded.

RESULTS

Figure 8 shows the effects of food intake at 2, 5 and 24 hr produced by AMPH (4 mg/kg), given alone or in combination with MORPH (1 mg/kg SC). The pattern of response to AMPH observed at both 2 and 5 hr was similar to that obtained in Experiment 1. Surprisingly, we found that 24-hr food consumption in AMPH-treated rats remained lower than in controls, even after tolerance had developed to the inhibition of the 5-hr food intake. Cumulating the quantity of food consumed during 9 days, a significant reduction in food intake was observed in the AMPH group (220.7±5.1 g compared to 257.3±9.8 g of food eaten by water-injected controls: −14.2%). The anorectic effects of AMPH were enhanced by MORPH, which, given alone, caused the expected

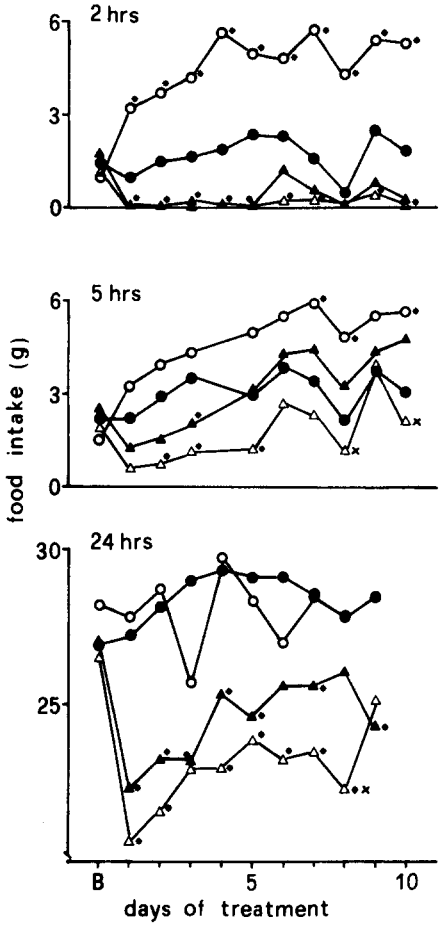

FIG. 8. Time course of the effects of 10-day AMPH administration alone (4 mg/kg IP, closed triangles) or in combination with MORPH (1 mg/kg given SC 10 min before AMPH; open triangles) on food intake at 2, 5 and 24 hr. Closed and open circles represent water and MORPH-injected controls, respectively. Each point represents the mean of six rats (SEM is omitted for the sake of clarity). $*p<0.05$ vs. water injected controls; $\times p<0.05$ vs. AMPH group (Duncan's test).

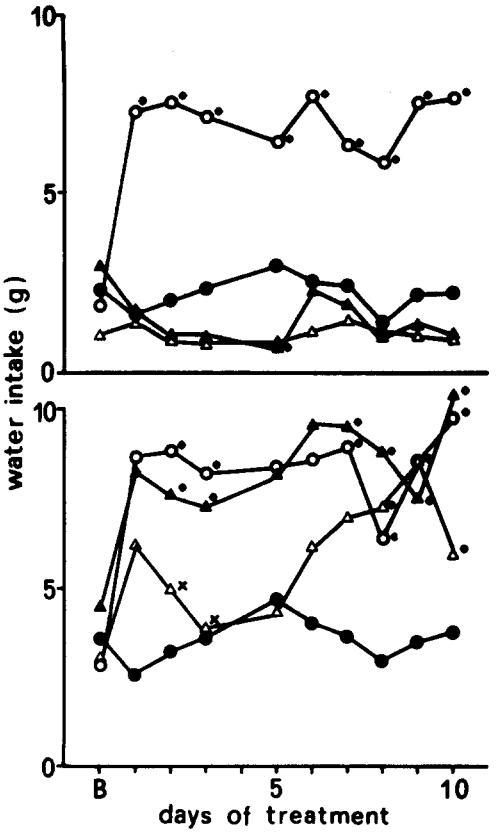

FIG. 9. Time course of the effects of 10-day AMPH administration alone or in combination with MORPH on water intake at 2 hr (upper panel) or 5 hr (lower panel). Same experiment and symbols as in Fig. 8.

activation of feeding restricted to the first 2 hr of observation. The enhancement of anorexia was small and at 5 hr only reached statistical significance on days 8 and 10. However it was consistent, food intake at 2 and 5 hr always being lower in the group treated with both MORPH and AMPH. The enhancement persisted at 24 hr and. 9-day cumulative food intake was 206.1 ±4.2, i.e., −18.2% less than in the MORPH-treated controls.

When the 5-hr water intake was considered, the enhancement of depressant effects of AMPH produced by MORPH was clearer (Fig. 9). Both drugs, given independently, produced a significant enhancement of water intake in comparison with controls. When AMPH and MORPH were given in combination, this enhancement was obtained on the first day only and was followed by a decline in drinking with a minimum on day 3. From day 6 on, water intake began to increase again and from day 8 it was again higher than in the controls. These changes in drinking did not seem to reflect modification in diuresis. Urine output was indeed increased in AMPH-treated group, but not in the MORPH group and the AMPH diuretic effect was seldom affected by MORPH administration (data not shown).

GENERAL DISCUSSION

Confirming previous reports [8,33] our study shows that chronic administration of high doses of AMPH produces remarkable modifications in ingestive behavior that extend well beyond the post-treatment period. We observed that long-term treatment with 4 mg/kg IP AMPH first depressed and then enhanced cumulaitve food intake during the 5 hr of post-injection observation. In addition, the interruption of AMPH administration produced a further increase in food ingestion that slowly extinguished during the post-treatment period. However, changes in food intake were not homogenously distributed during the 5 hr of observation and the enhancement was restricted to the last 3 hr, 2-hr food intake remained deeply depressed throughout the chronic treatment. This persistent inhibition, which has been observed in chronic treatment with both CATH or d-amphetamine [1,26], is considered the result of behaviors that disrupt feeding, such as hyperactivity and stereotypy, to which tolerance does not develop [6]. Indeed, during the entire treatment period AMPH-injected rats showed head "bobbing" which subsided about 2 hr after drug administration.

In a previous study we found that changes in ingestive behavior produced by chronic administration of CATH were associated with an enhancement of feeding stimulated by

U50, a selective kappa-opiate agonist [34]. The present study confirms these CATH effects and shows that they are also produced by chronic AMPH administration, suggesting that sensitization of U50 feeding activation by CATH is due to an amphetamine mechanism of action. The time course of this sensitization seems to overlap that of tolerance to the anorectic effect of AMPH. We found indeed that sensitization was not produced by a single AMPH dose; it was present after 9 AMPH administrations, when tolerance to the AMPH-mediated reduction of food intake at 5 hr had fully developed; it disappeared when AMPH treatment was discontinued, although the pattern of response obtained on day 1 was only reinstated 17 days after AMPH interruption. Such a time course of changes in the sensitivity to U50 effects appeared to be independent from manipulations associated with more than 5 weeks of treatment, as suggested by the crossover experiment performed 38 days after the beginning of treatment. The administration of U50 to the controls after AMPH produced the same response obtained on day 1 in the AMPH group, i.e., U50 slightly counteracted the anorectic effect of AMPH. Therefore the increased sensitivity to U50, as well as the tolerance to anorexia, appeared to be mostly under the control of pharmacological stimuli associated with chronic AMPH administration.

The selectivity of this sensitization was evaluated because the development of tolerance to the anorectic effects of amphetamine-like agents nonspecifically may allow the animals to become particularly sensitive to drugs that increase the reinforcing properties of food. Thus, we tested the influence of chronic AMPH administration on the effects of MORPH and DZP as representative of two classes of drugs (mu-opiate agonists and benzodiazepines, respectively) considered paradigmatic of pharmacological activation of ingestive behavior [4,22]. We found that MORPH-, but not DZP-mediated stimulation of food intake, was enhanced by chronic administration of AMPH, suggesting that sensitization involves opiate, but not GABA-ergic mechanisms. Since U50 is considered a very selective kappa-opiate agonist [34], and at the dose used (2 mg/kg, IP) MORPH is unlikely to have other effects than mu-mediated, the lack of selectivity in the sensitization between U50 and MORPH is an intriguing finding. The problem of which kind of opiate receptor is involved in feeding activation is in fact unsolved, mu- and kappa-opiates being equally efficient in stimulating ingestive behavior in a wide array of experimental conditions [22]. In our study we adopted the activation of urine output as a criterion of opiate selectivity, this effect being very sensitive to the action of kappa-opiate agonists [5]. As expected, sensitization to MORPH-mediated feeding produced by chronic AMPH administration was not associated with changes in urine output. However, even sensitization to U50 effects involved ingestive behavior alone, and diuretic response was stable, or even reduced. Taken together, these results suggest that, whichever receptor is involved, chronic AMPH administration produces a sensitization of the opiate mechanisms which regulate ingestive behavior and are consistent with the findings that both mu- and kappa-opiate mechanisms interact in catecholaminergic control of ingestive behavior [20, 23, 36]. However, it is difficult to explain how a functional sensitization could develop regardless of the kind of receptor involved. One possible interpretation is that AMPH is in fact able to increase sensitivity to both mu- and kappa-opiate mechanisms, but that they act at different points of the feeding behavior or on different macronutrients. If so, free access to a balanced diet, as in the experimental protocol adopted, cannot help to solve the issue.

Depression of the opiatergic stimulation of ingestive behavior seems to play a role in the anorectic effects of monoamine releasing agents. Chronic treatment with fenfluramine has been found to produce accumulation of hypothalamic endorphins, an effect interpreted as due to their decreased utilization and which suggests that the anorectic effects of fenfluramine may be mediated by an inhibition of the release of hypothalamic opioid peptides [11]. Chronic AMPH administration also produces an increase of hypothalamic endorphin content and this effect is associated with an enhanced sensitivity to naloxone-mediated anorexia [27]. As already outlined, cerebral endogenous opiates increase when their interaction with opiate receptors is hampered; this condition is also associated with receptor up-regulation and sensitization to the actions of exogenous opiates [31,32]. Accordingly, if AMPH inhibits opiate-mediated feeding mechanisms, it is plausible that its chronic administration brings about compensatory hypertrophy in these mechanisms. The abnormally high responses we obtained to the administration of exogenous opiates during chronic AMPH treatment are consistent with this view. However, we cannot rule out the possibility of a reverse sequence of events, i.e., that opiates produce feeding by altering a catecholaminergic system and that the enhanced response to these agents during chronic AMPH is a consequence of a sensitization (or a desensitization) of that system. The evidence that opioids can stimulate feeding by activating a hypothalamic alpha-adrenergic pathway which inhibits the activity of satiety neurons substantiates this alternative interpretation [20].

Of course, the appearance of hypersensitivity to opiate stimulation of feeding when tolerance to the anorectic effects of AMPH has developed is not evidence that the first is causal to the second. Looking for this causal link we gave AMPH and MORPH in combination for 10 days, in the assumption that maintaining the opiate tone could hinder the development of hypertrophy and hence the functional sensitization to opiates. As expected, during the experiment the lowest food intake at both 5 and 24 hr was in the group receiving AMPH and MORPH together, even though the MORPH dose adopted (1 mg/kg, SC) produced a remarkable increase of feeding when given alone. In addition, the inhibitory interaction between MORPH and AMPH also involved water intake. However, this potentiation cannot be only ascribed to a prevention of tolerance, because even acutely MORPH enhanced the anorectic effect of AMPH. Since MORPH has been found to increase stereotyped behavior produced by AMPH [35], the acute inhibitory interaction between the two drugs may be due to behavioral manifestations that are incompatible with food consumption. However, there is some evidence that besides stimulation, MORPH could produce inhibition of food intake [5]. Although this inhibition has been interpreted as a result of the sedative effects of high doses of MORPH [28], evidence of a more specific inhibitory role of MORPH in feeding has been provided by a study showing that MORPH restores the anorectic efficacy of fenfluramine in rats made tolerant to the action of this serotonergic agent [10]. In this context, our observation that hyperphagic effect of MORPH lasted 2 hr and was invariably followed by a remarkable reduction of food intake in the remaining 3 hr of observation could be interpreted as the result of an inhibitory mechanism. This interpretation is further supported by the recent finding that in freely-feeding rats, 2 hr of anorexia produced by naloxone is followed by 4 hr of high food intake [14].

Even if highly speculative, our interpretation of the MORPH-mediated increase of anorectic effect of AMPH is that ingestive behavior is under an opiatergic control which is both activatory and inhibitory. AMPH is able to inhibit the opiate activatory branch and to work synergically with the inhibitory branch. In this way, the acute interaction between MORPH and AMPH results in an additivity of their inhibitory effects. In addition, prolonged inhibition of the opiate activatory branch by chronic AMPH administration may produce its compensatory hypertrophy with the functional consequences of both a reduction in the anorectic effect of AMPH (i.e., tolerance) and a hypersensitivity to exogenous opiates.

This model, of course, does not fully explain modifications in ingestive behavior during chronic treatment with AMPH. In particular, throughout the experiment water and food ingestions revealed remarkable differences, although they seem to respond in the same way to the administration of either U50, MORPH, or DZP during chronic AMPH treatment. Thus, water intake, unlike food intake, was not inhibited in the initial days of AMPH treatment, and the interruption of AMPH administration did not lead to its overshoot, but to its progressive normalization. Closely similar results have been already found with chronic administration of d-amphetamine [33]. Since water intake is closely interconnected with both food intake and diuresis, we wondered if it covariated with them during AMPH treatment. As expected in rats [3], food and water intake at 5 hr were highly correlated in both the water- and AMPH-treated groups throughout the experiment. This correlation would suggest that the increase in drinking observed in the AMPH-treated group was a carry over effect of the enhanced food intake. However, the observation that polydipsia persisted even in conditions of food deprivation does not agree with this supposition. Thus we turned our interest to diuresis, which we confirmed to be stimulated by amphetamine-like agents [29]. Urine output and water intake were not usually correlated either in the water-treated group or, at the beginning of treatment, in the AMPH group. Prolonging AMPH treatment however resulted in highly correlated diuresis and drinking. Since urine output remained stable at high values, correlation could only be obtained by adjusting water intake on diuresis, as was the case. Therefore diuresis seems to be the physiological stimulus driving changes in water intake that appear during chronic AMPH treatment. This interpretation should not be challenged by the observation that the interruption of AMPH treatment brought about an immediate normalization of diuresis, whereas water intake remained higher than in the control group. It is indeed well known that physiological systems ruling hydric homeostasis are relatively sluggish in adapting to changes in electrolyte balance [16].

In conclusion, it is interesting to note that a syndrome characterized by polyuria, polydipsia and polyphagia is reminiscent of diabetes. In that disease, thirst is due to polyuria and is independent from hunger. Something like this should happen in animals made tolerant to AMPH and, if our interpretation is correct, sensitization of opiate mechanisms should play a role in polyphagia, whereas polydipsia should be under the control of polyuria. Of course, more study is needed to strengthen this interpretation.

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