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BRIEF COMMUNICATION

Environment-Specific Reinstatement of Amphetamine-Mediated Hyperdipsia by Morphine and (−)-Norpseudoephedrine

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NENCINI, P. AND S. FRAIOLI. *Environment-specific reinstatement of amphetamine-mediated hyperdipsia by morphine and (−)-norpseudoephedrine.* PHARMACOL BIOCHEM BEHAV 47(2) 339–343, 1994.—In a study designed to determine whether environmental and pharmacological stimuli have the ability to take control of amphetamine-mediated hyperdipsia, rats were injected with *d,l*-amphetamine (AMPH; 4 mg/kg, IP) alone or in combination with (−)-norpseudoephedrine (NPE; 10 mg/kg, IP) and then returned to the home cage or transferred to a distinct environment (test cage). Water intake was measured hourly for 3 h, in the absence of food. AMPH treatment lasted for 10 days, followed by a 6-day extinction phase during which AMPH, but not NPE, injections were discontinued. Subsequently, all animals received challenge injections: NPE (10 mg/kg) on day 17; AMPH (4 mg/kg) on day 19; and morphine (MOR; 1 mg/kg) on day 21. AMPH-mediated hyperdipsia developed in 50% of animals and had an early onset in the home cage. NPE prevented the AMPH effects. Discontinuation of AMPH treatment promptly normalized drinking in the home cage but increased it further in the test cage. Within 6 days of AMPH discontinuation, hyperdipsia completely disappeared. It was reinstated, in the test cage alone, by a challenge injection of NPE or MOR. We suggest that hyperdipsia is a primary AMPH effect, which in some way is counteracted by a distinct environment. This appears to elicit a compensatory mechanism that is revealed in the absence of AMPH and is reinstated in a nonspecific way by pharmacological stimuli.

d,l-Amphetamine (−)-Norpseudoephedrine Morphine Hyperdipsia Drinking

CHRONIC intermittent administration of moderate doses of amphetamine (AMPH) produces an increase in water intake, which becomes apparent 2 h after drug administration, lasts no more than 5 h, and leaves daily water intake unchanged (4,6,14,15,20,22). Because it develops and is maintained in the absence of food, amphetamine-induced hyperdipsia does not depend on food intake (14,16). Nor is it a secondary consequence of increased salt or water loss because it develops when the diuretic effect of amphetamine is suppressed (17).

Whereas cathinone, a potent amphetamine-like agent, produces hyperdipsia (15), (−)-norpseudoephedrine (NPE), a weak sympathomimetic drug, does not (17). Thus, hyperdipsia appears to develop only in response to drugs with full amphetamine-like properties. Despite this, once produced, hyperdipsia may be maintained or reinstated by weaker agents. Such a hypothesis is indirectly supported by evidence that drugs pharmacologically related to psychomotor stimulants, but seldom abused, reinstate the intake of more powerful compounds. Bromocriptine, for instance, reinstates cocaine self-administration in rats (24) and desipramine treatment causes postaddicts to relapse into cocaine abuse (23). A parsimonious explanation of this interaction is that the stimulus properties of the weaker drug could signal the effects of the more powerful agent. Desipramine-induced jitteriness may thus have triggered the relapse of cocaine abuse (23). In comparison to AMPH, NPE has much weaker effects on locomotion, feeding, and thermogenesis (2,3,8) and at IP doses of 15 and 30 mg/kg does not stimulate hyperdipsia (17). This makes NPE particularly suitable for evaluating the ability of a mild sympathomimetic agent to take control of the expression of hyperdipsia produced by chronic AMPH. For this purpose, we studied the effects of NPE under extinction conditions by testing the efficacy of the drug in maintaining hyperdipsia in rats chronically treated with both AMPH and NPE and in

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reinstating hyperdipsia in AMPH-treated controls. We evaluated these hyperdipsic responses in two distinct environments: the home cage and the test cage. This comparison was motivated by our previous observation that hyperdipsia developed more slowly in the test cage (4). If this slowing depended on an AMPH effect that competed with drinking, we predicted that AMPH discontinuation would produce a rebound hyperdipsia enhanced by NPE.

METHOD

Animals

Subjects were 72 male Sprague–Dawley rats (Morini, San Polo D'Enza, RE, Italy) with an average weight of 320 g at the beginning of the study. On their arrival in the laboratory, rats were initially housed in small groups and then kept singly in the home cages at 21 ± 2°C, with a daylight cycle (the length of the light phase ranged from 11 to 15 h and the onset between 4:40 and 6:40 a.m.). During this period, rats had free access to food and water and were allowed to adapt to the new environment; manipulations were restricted to a daily handling for recording weight.

Apparatus and Procedure

The home cages, which measured 27 × 21 × 14 cm, had a Plexiglas floor and rats were in direct contact with the sawdust. The test cages, unlike the home cages, had a wire grid floor (1.1 cm^2 mesh). Nondeprived rats were used for the experiments. After 3 days of housing, animals were randomly assigned to two groups. After injection, rats in the first group were immediately returned to their individual home cages and those in the second group to the test cages. In both conditions and for the following 3 h, rats had access to tapwater but not to food.

After 7 days of baseline (IP injection of water and relevant experimental procedure, see below), the home-cage and test-cage groups were subdivided into four groups of nine rats. Animals received IP water or NPE (10 mg/kg) 15 min before water or AMPH (4 mg/kg), according to a 2 × 2 design representing all treatment combinations. AMPH treatment lasted 10 days and thereafter it was substituted by a daily solvent injection. NPE treatment continued for a further 11 days. All animals received a challenge injection: NPE (10 mg/kg) on day 17, AMPH (4 mg/kg) on day 19, and MOR (1 mg/kg) on day 21.

During the test, rats had no access to food; water intake was measured by weighing the bottles before and 1, 2, and 3 h after drug administration. The maximal spillage produced by weighing the bottles was 0.1 g and this quantity was accounted for.

Drugs

d,l-AMPH monobasic racemic (BDH, Poole, UK) was dissolved in diluted hydrochloric acid and the solution brought to neutrality by adding diluted alkali (final volume: 1 ml/kg). (−)-Norpseudoephedrine HCl (EGA-Chemie, Steinheim/Albuch, Germany) and morphine sulphate (SALARS, Como, Italy) were freshly dissolved in distilled water to a final volume of 1 ml/kg.

Data Analysis

To reduce intrasubject variability, the data were averaged over blocks of 2 consecutive days. Data were processed using an analysis of variance (ANOVA) with three between factors (AMPH, NPE, and cage) and two within factors (days and hours). Tukey's test was used for posthoc group comparisons.

Although ANOVA yielded a significant AMPH effect at 2 and 3 h during the 10 days of treatment, the raw data clearly showed that only some AMPH-treated rats developed hyperdipsia. To provide an objective distinction between hyperdipsic (responders) and normo- or hypodipsic (nonresponders) rats, as a criterion of hyperdipsia we adopted values of water intake above the upper 5% confidence limit of the respective control mean for 8 of 10 days (four blocks) or, alternatively, for the last 6 days of treatment (the last three consecutive blocks). Five of the nine home-cage and four of the nine test-cage animals met the criterion. In NPE- and AMPH-treated groups, a total of three animals met the criterion. Two of them (test-cage condition) showed baseline drinking levels above the confidence limit and were therefore omitted from statistical analysis, as was the only rat that showed hyperdipsia in the home cage.

RESULTS

All rats, except two that had to be killed because of respiratory distress, remained healthy throughout the experiment. AMPH-treated rats gained weight more slowly than controls [ANOVA on body weight gain difference between the tenth and the first treatment day showed a significant AMPH effect, $F(1, 59) = 19.95$, $p = 0.001$]. But, their body weight increased faster during the posttreatment period, $F(1, 59) = 5.10$, $p = 0.028$. These effects of AMPH on body weight were influenced neither by NPE nor by testing rats in a separate cage.

Drinking During AMPH Treatment

During the first hour after drug administration, AMPH significantly inhibited drinking, $F(1, 59) = 12.46$, $p = 0.001$. However, a progressive increase in water intake in the responders group was noticeable from days 7 to 8, particularly in the test cage. This may account for the significant interaction between AMPH, cage, and days factors, $F(4, 236) = 2.73$, $p = 0.030$. Despite the small dose administered, NPE significantly reduced water intake, $F(1, 59) = 11.96$, $p = 0.001$ (data not shown).

During the subsequent 2 h, AMPH significantly enhanced water intake, $F(1, 59) = 7.87$, $p = 0.007$ (Fig. 1). This AMPH effect developed faster in the home cage and on days 5–6 the water intake of responders differed significantly in the two environmental conditions. Although by itself NPE did not significantly influence water intake, it prevented the development of AMPH-induced hyperdipsia in the home cage [AMPH × NPE, $F(1, 59) = 5.03$, $p = 0.029$].

Drinking During Post-AMPH Treatment

Substitution of a water injection for AMPH caused little change in the water intake at 1 h in the AMPH groups maintained in the home cage. In contrast, water intake in the test-cage responders group showed a remarkable increase, which was prevented by continuing NPE treatment [NPE factor at 1 h, $F(1, 59) = 8.62$, $p = 0.005$] (Fig. 2). During the following 2 h (data not shown), the influence of the cage factor on drinking became statistically more evident, $F(1, 59) = 6.14$, $p = 0.016$, whereas NPE inhibition of drinking disappeared, leaving uncontrasted the increased water intake in groups pretreated with AMPH, $F(1, 59) = 7.04$, $p = 0.01$.

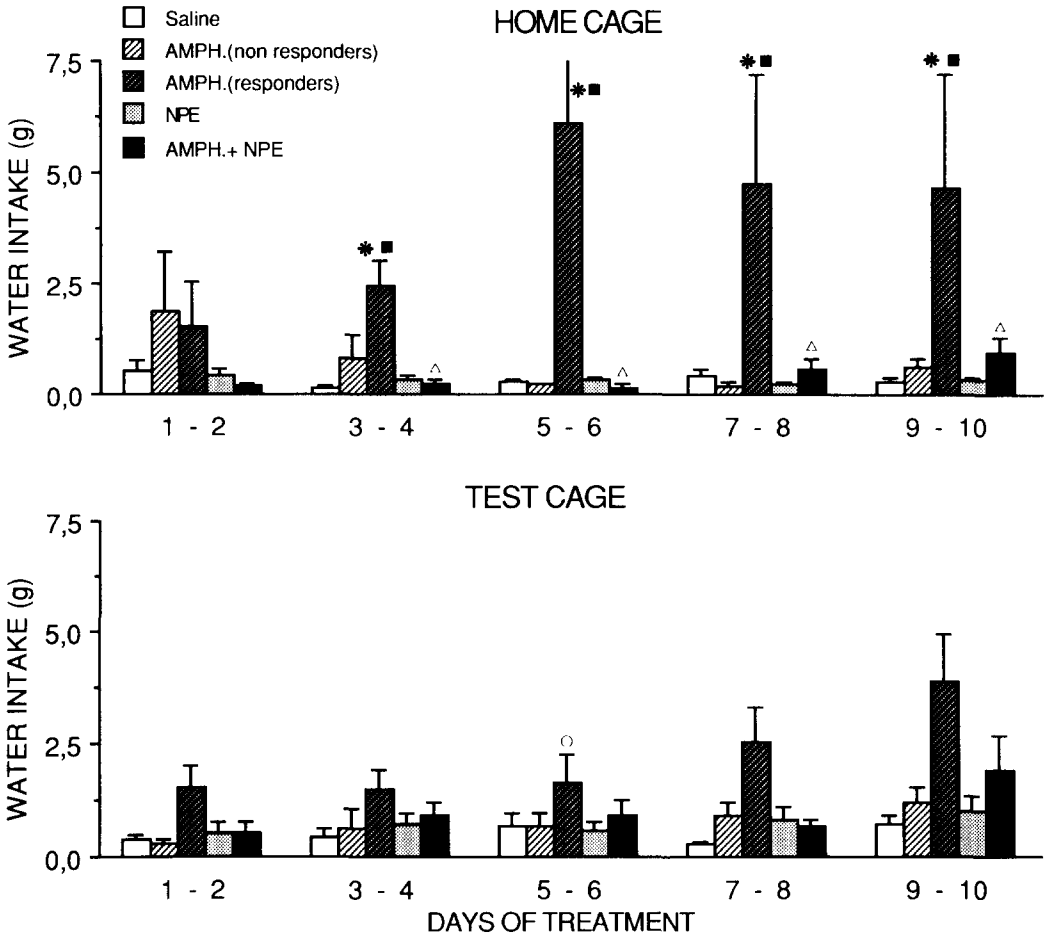

FIG. 1. Cumulative water intake at 2 and 3 h. Water intake in the home cage (top) or in the test cage (bottom) after the daily IP injection of amphetamine (AMPH) given alone (responders: dark hatched bars, $n = 5$ in the home cage and $n = 4$ in the test cage; nonresponders: light hatched bars, $n = 4$ in the home cage and $n = 5$ in the test cage) or in combination with (−)-norpseudoephedrine (NPE) (dark dotted bars, $n = 7$ in both the home- and test-cage groups). Open bars represent groups treated with the solvent ($n = 8$ in the home cage and $n = 9$ in the test cage) and light dotted bars those with NPE ($n = 9$ in both the home- and test-cage groups). Each point represents the mean of 2 consecutive days. (*), $p < 0.05$ vs. solvent group; (■), $p < 0.05$ vs. nonresponders group; (△), $p < 0.05$ vs. responders group; (○), $p < 0.05$ vs. home-cage responders group; Tukey's test.

Reinstatement of Drinking Response

Figure 2 also shows the results obtained by injecting NPE, AMPH, or MOR to all groups on days 17, 19, and 21, respectively. The drinking pattern during the first hour after NPE administration paralleled that observed at the beginning of the post-AMPH-treatment phase. NPE reinstated hyperdipsia in the responders group, but did so only in the test cage.

The challenge injection of AMPH elicited a drinking response at 2–3 h that largely reproduced the differences between responders and nonresponders observed during AMPH treatment. However, AMPH also produced an unexpected increase in water intake in the test-cage water-injected controls. Likewise, two of the NPE controls (4.7 and 9.6 ml) and one of the NPE–AMPH-treated rats (6.7 ml) exhibited remarkable hyperdipsic responses to AMPH, whereas all other animals in these two groups drank less than 1 ml throughout the 3 h. Owing such a large variance, differences among groups did not reach significance.

Although adopted as a negative control, MOR was able to reinstate statistically significant differences between the water intake in responders and nonresponders. These differences were prevented by NPE administration and, again, were elicited in the test cage only.

DISCUSSION

Although the present study focused on the question of whether a light sympathomimetic drug could take control of the hyperdipsic response to AMPH in rats, the large individual differences in such a response merit preliminary discussion. Adopting as a parsimonious criterion for hyperdipsia a level of drinking over the upper limit of control confidence interval for 8 of 10 days of treatment or, alternatively, for the last 6 days, we observed that only half the rats treated with AMPH became hyperdipsic. A retrospective analysis of our previous experiments shows that these differences are reproducible (6,14,16,17). The same AMPH dose (4 mg/kg) and a slightly prolonged observation of postinjection drinking (i.e., 5 h) caused a responders rate of 65%. At the dose of 2 mg/kg,

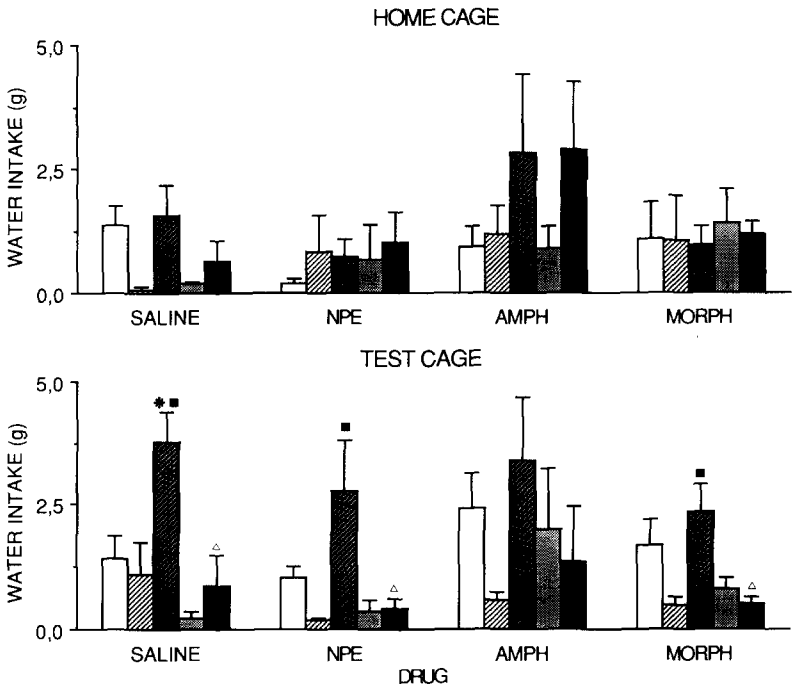

FIG. 2. Water intake at 1 h during the first day of postamphetamine (AMPH) treatment and after a challenge injection of (−)-norpseudoephedrine (NPE), AMPH, or morphine (MORPH). Water intake in the home cage (above) or in the test cage (below) after the daily IP injection of AMPH, but not of NPE, had been substituted by a solvent injection (saline, days 11–12) or by an injection of NPE (10 mg/kg, day 17), AMPH (4 mg/kg, day 19), or MORPH (1 mg/kg, day 21). Figures represent water intake at 1 h after saline or NPE and at 2 and 3 h after AMPH and MORPH injections. Same symbols as in Fig. 1.

the rate dropped to 13.3%, suggesting that recruitment of responders is a dose-dependent phenomenon. We are not aware of other studies showing such striking individual differences in sensitivity to the hyperdipsic effect of AMPH. But, these differences are not surprising in view of individual differences in sensitization to the locomotor response to AMPH (10,18), differences that are abolished by increasing the dose of the drug (10). In these studies, high and low responders differ in other behavioral responses, including locomotion in a novel environment and vulnerability to AMPH self-administration (7,1,18).

Drinking differences between responders and nonresponders were influenced by environmental as well as pharmacological stimuli and outlasted AMPH treatment. In particular, during the posttreatment phase in the test cage water intake was higher in responders than in nonresponders, a difference that was enlarged by a challenge injection of NPE. This finding provides further support for the notion that AMPH is a stimulus capable of selecting subjects with a different ability to develop hyperdipsia. In addition, it seems to confirm the working hypothesis of this study that a light sympathomimetic agent can take control of AMPH-induced hyperdipsia. But, the interaction between NPE and AMPH on drinking proved to be complex. When NPE was associated with AMPH, the rate of rats developing hyperdipsia dropped from 50% to 6% (1/16). The dose we used, 10 mg/kg, was far below the lowest limit of NPE efficacy in suppressing feeding (2,3). However, in a previous study chronic administration of 15 or 30 mg/kg NPE prevented the increased fluid intake elicited by substituting a 6% alcohol solution for tapwater (16). Thus, doses of NPE that probably lack stimulant properties inhibit the acquisition of a hyperdipsic behavior in response to pharmacological and taste stimuli or both. Whether this inhibition results from taste aversion or from a primary antidipsic action is not clear, taking into account evidence that sympathomimetics share both mechanisms of drinking inhibition (9,12,21). Nevertheless, the possibility that NPE exerted a primary antidipsic action is weakened by the observation that, after AMPH discontinuation, NPE reinstated hyperdipsia in responders.

NPE not only reinstated hyperdipsia, it also reproduced the AMPH-mediated difference in water intake between responders and nonresponders, a difference that extinction had abated. This finding suggests that NPE has the stimulus properties needed to take control of the drinking response to AMPH. Yet, these stimulus properties are unlikely to include sympathomimetic activation because MOR was also able to reinstate hyperdipsia in responders. In addition, the reinstatement of AMPH-mediated hyperdipsia by NPE and MOR depended on the pairing of drugs with a specific environment, that is, NPE and MOR were active only in animals tested in a separate cage.

When AMPH was administered again, the effects of the drug on drinking were concealed by an unexpected intragroup scattering of water intake values. This scattering was particularly wide both within the groups in which NPE administration was associated with the home or the test cage and within

the test cage control group. Chronic exposure to NPE or to a distinct environment might have sensitized potential responders to the hyperdipsic challenge of AMPH. This speculation would be consistent with the finding of Antelman et al. that a nonspecific stressor (acidic vehicle injection) produces a long-lasting sensitization of rats to the hyperdipsic effect of AMPH (1).

We provisionally interpret the environment-specific capability of NPE and MOR to reinstate hyperdipsia in the context of adaptative mechanisms. We propose that hyperdipsia in the home cage arises from a process of sensitization to AMPH (5,19). Although cross-sensitization between potent psychomotor stimulants has been observed (11,13), at the dose used NPE is probably too weak to elicit a response in rats sensitized to AMPH. Strongly in favor of this explanation is NPE's failure to potentiate and maintain AMPH-mediated hyperdipsia. The lack of effects of MOR in the home cage further confirms that the reinstatement of hyperdipsia in the familiar environment requires a direct amphetamine-like mechanism.

The hyperdipsia observed in the test cage during the extinction phase would be the result of compensation for AMPH effect(s) that constrains the development of hyperdipsia. If this hypothesis is correct, the residual AMPH-like properties of NPE would alone be sufficient to elicit a compensatory hyperdipsia conditioned to the AMPH administration. The efficacy of MOR implies that the reinstatement of hyperdipsia does not require the presence of specific pharmacological stimuli. This is not surprising. Also under conditions of self-administration, the ability of agents to reinstate responses after a period of extinction shows a low degree of pharmacological specificity (24). Antelman has suggested that "the sensitization properties of a drug are less a function of its specific pharmacological effects than of its nonspecific stress effects by virtue of being a foreign substance" (1). In this conceptual framework, our study suggests that being a foreign substance may also suffice to reinstate compensatory responses generated by a different pharmacological stimulus.

REFERENCES

1. Antelman, S. M.; Caggiula, A. R.; Knopf, S.; Kocan, D. J.; Edwards, D. J. Amphetamine or haloperidol 2 weeks earlier antagonized the plasma corticosterone response to amphetamine; evidence for the stressful/foreign nature of drugs. Psychopharmacology (Berl.) 107:331–336; 1992.
2. Arch, J. R. S.; Ainsworth, A. T.; Cawthorne, M. A. Thermogenic and anorectic effects of ephedrine and congeners in mice and rats. Life Sci. 30:1817–1826; 1982.
3. Blosser, J. C.; Barrantes, M.; Parker, R. B. Correlation between anorectic potency and affinity for hypothalamic (+)-amphetamine binding sites of phenylethylamines. Eur. J. Pharmacol. 134: 97–103; 1987.
4. Camanni, S.; Nencini, P. Physiological and behavioral aspects of drinking stimulated by chronic exposure to amphetamine in rats. Gen. Pharmacol.; in press.
5. Castaneda, E.; Becker, J. B.; Robinson, T. E. The long-term effects of repeated amphetamine *in vivo* on amphetamine, KCl and electrical stimulation evoked striatal dopamine release *in vitro*. Life Sci. 42:2447–2456; 1988.
6. Consorti, D.; Nencini, P. Chronic yohimbine prevents the anorectic effects of amphetamine in rats. Abstracts of the Third IBRO World Congress of Neuroscience, August 4–9, 1991, Montreal, Canada, p. 273.
7. Deminière, J. M.; Piazza, P. V.; Le Moal, M.; Simon, H. Experimental approach to individual vulnerability to psychostimulant addiction. Neurosci. Biobehav. Rev. 13:141–147; 1989.
8. Fairchild, M. D.; Alles, G. A. The central locomotor stimulatory activity and acute toxicity of the ephedrine and norephedrine isomers in mice. J. Pharmacol. Exp. Ther. 158:135–139; 1967.
9. Guaza, C.; Borrell, S.; Borrell, J. Effects of adrenaline on the acquisition and maintenance of ethanol preference in a taste conditioning paradigm. Psychopharmacology (Berl.) 90:336–340; 1986.
10. Hooks, M. S.; Jones, G. H.; Neill, D. B.; Justice, J. B. Individual differences in amphetamine sensitization: Dose-dependent effects. Pharmacol. Biochem. Behav. 41:203–210; 1991.
11. Horger, B. A.; Giles, M. A.; Schenk, S. Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology (Berl.) 107:271–276; 1992.
12. Hunt, T.; Amit, Z. Conditioned taste aversion induced by self-administered drugs: Paradox revisited. Neurosci. Biobehav. Rev. 11:107–130; 1987.
13. Kolta, M. G.; Shreve, P.; Uretsky, N. J. Effect of methylphenidate pretreatment on the behavioral and biochemical responses to amphetamine. Eur. J. Pharmacol. 117:279–282; 1985.
14. Nencini, P. The role of opiate mechanisms in the development of tolerance to the anorectic effects of amphetamines. Pharmacol. Biochem. Behav. 30:755–764; 1988.
15. Nencini, P.; Johanson, C. E.; Schuster C. R. Sensitization to kappa opioid mechanisms associated with tolerance to the anorectic effects of cathinone. J. Pharmacol. Exp. Ther. 245:147–154; 1988.
16. Nencini, P.; Valeri, P. (−)-Norpseudoephedrine, but not (±)-amphetamine, prevents the increase in fluid intake associated with ethanol presentation in rats. Pharmacol. Res. 24:83–91; 1991.
17. Nencini, P.; Valeri, P.; Morrone, L. A. Dapiprazole, a selective alpha-1 adrenoceptor antagonist, inhibits diuresis but not polydipsia produced by amphetamine in rats. Brain Res. Bull. 25:765–767; 1990.
18. Piazza, P. V.; Deminière, J.-M.; Le Moal, M.; Simon, H. Factors that predict individual vulnerability to amphetamine self-administration. Science 245:1511–1513; 1989.
19. Robinson, T. E.; Becker, J. B. Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev. 11:157–198; 1986.
20. Rowland, N.; Antelman, S. M.; Kocan, D. Elevated water intake in rats treated chronically with amphetamine: Drinking in excess of need? Appetite 2:51–66; 1981.
21. Stolerman, I. P.; D'Mello, G. D. Amphetamine-induced hypodipsia and its implications for conditioned taste aversion in rats. Pharmacol. Biochem. Behav. 8:333–338; 1978.
22. Tormey, J.; Lasagna, L. Relation of thyroid function to acute and chronic effects of amphetamine in the rat. J. Pharmacol. Exp. Ther. 128:201–209; 1960.
23. Weiss, R. D. Relapse to cocaine abuse after initiating desipramine treatment. JAMA 260:2545–2546, 1988.
24. Wise, R. A.; Murray, A.; Bozarth, M. A. Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained lever pressing in rats. Psychopharmacology (Berl.) 100:355–360; 1990.