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Tolerance Does Not Develop to the Suppressant Effects of (−)-Norpseudoephedrine on Ingestive Behavior in the Rat

P. NENCINI,[1] S. FRAIOLI AND D. PERRELLA

Institute of Medical Pharmacology, University of Rome "La Sapienza," P.le A. Moro 5, 00185 Rome, Italy

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NENCINI, P., S. FRAIOLI AND D. PERRELLA. *Tolerance does not develop to the suppressant effects of (−)-norpseudoephedrine on ingestive behavior in the rat.* PHARMACOL BIOCHEM BEHAV **53**(2) 297–301, 1996.—(−)-Norpseudoephedrine (NPE), the enantiomer of cathine and a structural analog of phenylpropanolamine, shows anorectic and antidipsic effects that have been referred to its structural analogies with amphetamine. When amphetamine is chronically administered to rats, its anorectic effects fade out, water intake is progressively increased, and the diuretic response to the drug remains stable. Our previous studies show that chronic administration of NPE does not produce the typical amphetamine hyperdipsic response. In the present study, designed to obtain a more detailed picture of the ingestive and diuretic effects of chronic exposure to NPE, we evaluated the effects of 11 daily administrations of three doses of NPE (17, 32, and 56 mg/kg IP) on food and water intake, as well as on diuresis, in rats maintained in conditions of free access to food and water. Results show that all three doses inhibited food intake at 2 h, whereas only the highest dose inhibited food intake at 5 h. No differences between groups were detected at 24 h. These responses remained unchanged throughout the 11 days of treatment, and substitution of NPE with a solvent injection caused no rebound feeding. NPE treatment did not modify the ingestive response to a challenge injection of amphetamine, 0.56 and 1.0 mg/kg IP, given 1 day apart. Although NPE inhibited water intake throughout the experiment, it did so significantly only during the first 2 h postinjection. Urine output in the NPE-treated groups increased significantly on the first day only. These findings make it unlikely that the anorectic effects of NPE depend on an amphetamine-like mechanism of action. In addition, the short-lasting anorectic and antidipsic effects of NPE and the lack of tolerance to them raise the possibility of a therapeutic use of this drug as an adjuvant in the therapy of eating disorders characterized by binge episodes.

(−)-Norpseudoephedrine *d*-Amphetamine Food intake Water intake Anorexant khat

THE SIDE chain of phenylethylamine contains two asymmetric atoms of carbon that generate two couples of enantiomers. One couple consists of [1R,2R]-(−)-norephedrine and [1S,2S]-(+)-norephedrine, and its racemic mixture is termed phenylpropanolamine. The other two enantiomers are [1R,2S]-(−)-norpseudoephedrine and [1S,2R]-(+)-norpseudoephedrine. The latter, better known as cathine, is one of the active principles of *Catha edulis*. Phenylpropanolamine is a mild sympathomimetic agent and is widely popular as a component of several over-the-counter cough-, cold-, and appetite-suppressant remedies (9). Because of its amphetamine-like psychomotor stimulant effects, cathine has been included in the list of potentially addictive drugs (7,11). The scanty literature dedicated to [1R,2S]-(−)-norpseudoephedrine (NPE) has been mainly addressed to a comparative analysis of the effects of phenylethylamines on food intake in rodents. These studies show that in free-feeding normal and obese mice NPE inhibits daily food intake and slightly increases energy expenditure (1). Four weeks of treatment extinguish the anorectic effect of NPE in obese, but not in lean mice. The anorectic actions of NPE have been confirmed in food-restricted rats, where its acute administration reduces the size of a meal in a dose-dependent way (3). The evidence that NPE activates locomotion suggests that this compound preserves some of the amphetamine-like properties shown by its enantiomer cathine (5). Whether these supposed residual amphetamine-like properties are responsible for the anorectic effects of NPE is less certain. Phenylpropanolamine, for instance, stimulates locomotion

[1] To whom requests for reprints should be addressed.

and generalizes for amphetamine in drug-discrimination paradigms (5,8), but it does not seem to share the amphetamine-like mechanism of anorectic action (18).

We have found that NPE and amphetamine differ remarkably in their effects on fluid ingestion in the rat. Chronic daily injections of moderate doses of amphetamine are known to produce a progressive increase in water intake between 2 and 5–7 h after drug administration (10,12,14,15). NPE does not produce this hyperdipsic response, but, in contrast, it prevents that elicited by amphetamine (12). Another difference is that chronic NPE, but not chronic amphetamine, prevents the increased fluid intake produced by substituting water with a 6% ethanol solution (13).

In the present study, we further explored the ingestive effects of chronic daily administrations of NPE by measuring food and water intake at 2, 5, and 24 h after drug administration in rats maintained in conditions of free access to food and water. These experimental conditions make it possible to distinguish between the early (2 h) inhibitory and late (5 h) activatory actions of moderate doses of amphetamine (3–4 mg/kg IP) (10,14). Whereas the early inhibition is not modified by chronic administration of the drug, the late activation is progressively enhanced over time (sensitized) [see, for instance, (2,14)]. As a further element of comparison with the amphetamine effect, we measured urine output, because amphetamine-induced diuresis leads neither to tolerance nor to sensitization (10).

METHOD

Animals

Twenty-four male Sprague–Dawley rats (Morini, San Polo d'Enza) with weights ranging from 275 to 326 g at the beginning of the study were used for the experiments. The animals were housed singly in metabolic cages (Tecniplast Gazzada) at 23°C with a 12 L : 12 D cycle (0700–1900 h). They had free access to water and food, the latter being available as a gross powder, obtained by grinding lab pellets (Standard Diet 4RF21, Mucedola s.r.l.). To minimize spillage due to behaviors not related to food intake (i.e., stereotyped gnawing) we avoided dispensing lab pellets. During the first week, in which the rats were allowed to adapt to the new environment, manipulation was restricted to a daily handling for weight record. For the next 3 days before drug treatment began the animals were injected intraperitoneally with water, and independent measures were taken as described below.

Independent Measures

During the experimental procedure, food and water intake and urine output were measured by weighing (to the nearest 0.1 g) food receptacles, water bottles, and urine cylinders before and 2, 5, and 24 h after drug administration. To prevent evaporation, urine cylinders contained a layer of mineral oil.

Procedure

Animals were randomly distributed in four groups, which were then injected daily with solvent or three different doses of NPE, already found to be active in affecting feeding and drinking [17, 32, and 56 mg/kg IP, respectively; see (1,3, 12,13)]. Solvent or drug were injected early during the daily light period (usually at 0900 h). NPE treatment lasted 11 days and was then substituted by a solvent injection for 2 days. On day 14, all the groups were injected with 0.56 mg/kg *d*-amphetamine, and on day 15 with 1.0 mg/kg. The rationale for this procedure is that discontinuation of chronic amphetamine administration elicits an overshoot of food intake and an apparent normalization of water intake, associated with a long-term change in the feeding and drinking responses to psychomotor stimulants and opioid drugs (2,10,12). Independent variables across the 15 days of the experiment were measured as described.

Data Analysis

Data were processed using an analysis of variance (ANOVA) with one between factor (NPE: four levels) and one within factor (days: 11 levels). Tukey's test was used for subsequent comparisons within logical sets of means. In order to have a more synthetic view of the time course of NPE effects on ingestive behavior, cumulative food and water intake at 2, 5, and 24 h was averaged across 11 days of treatment and a one-way ANOVA (NPE: four levels) was performed for each time interval. The effect of NPE was then shown (Fig. 3) as a percentage of the control response.

Drugs

(−)Norpseudoephedrine (threo-2-amino-1-hydroxy-1-phenylpropane) (Sigma Chemical Co, St. Louis, MO) and S(+)-amphetamine sulfate (Research Biochemical International, Natick, MA) were freshly dissolved in distilled water to a final volume of 1 ml/kg.

RESULTS

Food Intake

NPE remarkably inhibited food ingestion. Inhibition peaked during the first 2 h, and in the group treated with the highest NPE dose (56 mg/kg IP) persisted during the following 3 h (Fig. 1). ANOVA shows a significant drug effect at 2 h, $F(3, 19) = 22.981$, $p < 0.001$). Despite a significant within-subjects effect [days of treatment: $F(10, 190) = 9.521$, $p < 0.001$], post hoc analysis failed to detect a trend in the anorectic effect of NPE. The effect of the drug persisted during the following 3 h, $F(3, 20) = 6.376$, $p = 0.003$, but the post hoc analysis revealed that this depended mainly on the inhibitory effect of the highest NPE dose (56 mg/kg IP). Again, a significant within-subject effect, $F(10, 200) = 1.993$, $p = 0.036$, did not correspond to a clear trend throughout days of treatment. A visual inspection of the gross behavior during the first 5 h shows that at the highest doses (32 and 56 mg/kg) NPE produced some motor activation, consisting of locomotion and head bobbing. In contrast, the lowest dose produced no motor effects. During the rest of the day (5 to 24 h), NPE-treated groups ate more than controls [drug effect: $F(3, 20) = 4.837$, $p = 0.011$], but post hoc analysis detected no dose-related effect.

Averaging the cumulative food intake at 2, 5, and 24 h across the 11 days of treatment disclosed a clear dose-related suppression of food intake at both 2 and 5 h (Fig. 3, upper panel). After the highest NPE dose, a slight, but nonsignificant decrease in feeding could still be detected at 24 h.

Substituting solvent for NPE caused no rebound effect on feeding (Fig. 1). Likewise, controls and NPE-pretreated groups had statistically overlapping feeding responses to the administration of amphetamine 0.56 or 1.0 mg/kg IP.

Water Intake

Drinking appeared to be more resistant to the inhibitory effects of NPE, a significant inhibitory drug effect being de-

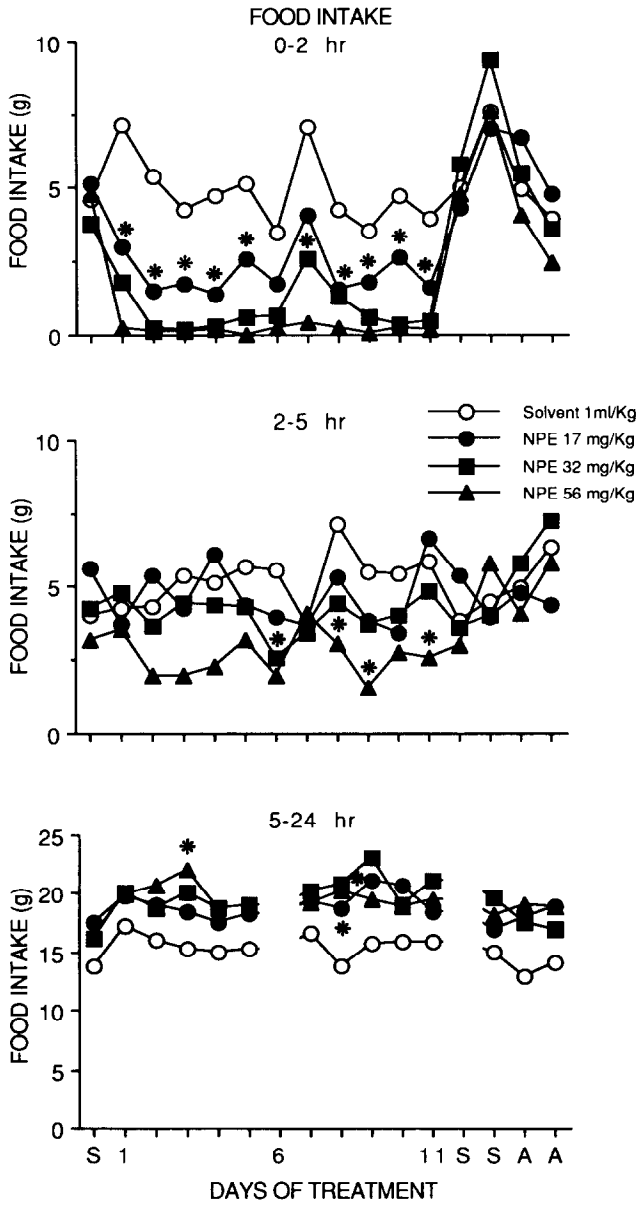

FIG. 1. Mean fractional food intake at 2, 5, and 24 h in groups treated with solvent or with different doses of NPE. The symbol S in abscissa indicate the administration of solvent to all the groups; the two A indicate that *d*-amphetamine was administered at the dose of 0.56 mg/kg (the first day) and 1.0 mg/kg (the second day). For the sake of clarity, SEM bars have been omitted. For the same reason statistical significance is shown only for the dose effect closest to the solvent effect. $*p < 0.05$ vs. solvent group; Tukey's test.

tected at 2 h, $F(3, 18) = 23.383$, $p < 0.001$, but not during the following 3 h, $F(3, 19) = 2.287$, $p = 0.111$ (Fig. 2). No significant within-subject effect occurred at either interval, suggesting that the effects of NPE on water intake remained stable across the 11 days of treatment. During the rest of the day water intake increased significantly in all the NPE groups, $F(3, 20) = 3.591$, $p = 0.032$, so that NPE administration did not change the cumulative daily water intake was (Fig. 3, lower panel).

No differences were observed between NPE-pretreated groups and controls when NPE was substituted with solvent. The injection of *d*-amphetamine 0.56 mg/kg or 1.0 mg/kg to all the groups failed to disclose a difference between NPE-treated animals and controls.

Body Weight

The ingestive effects of NPE left body weight unchanged throughout the 11 days of treatment, as suggested by the lack of significance for the drug factor both between subjects, $F(3,$

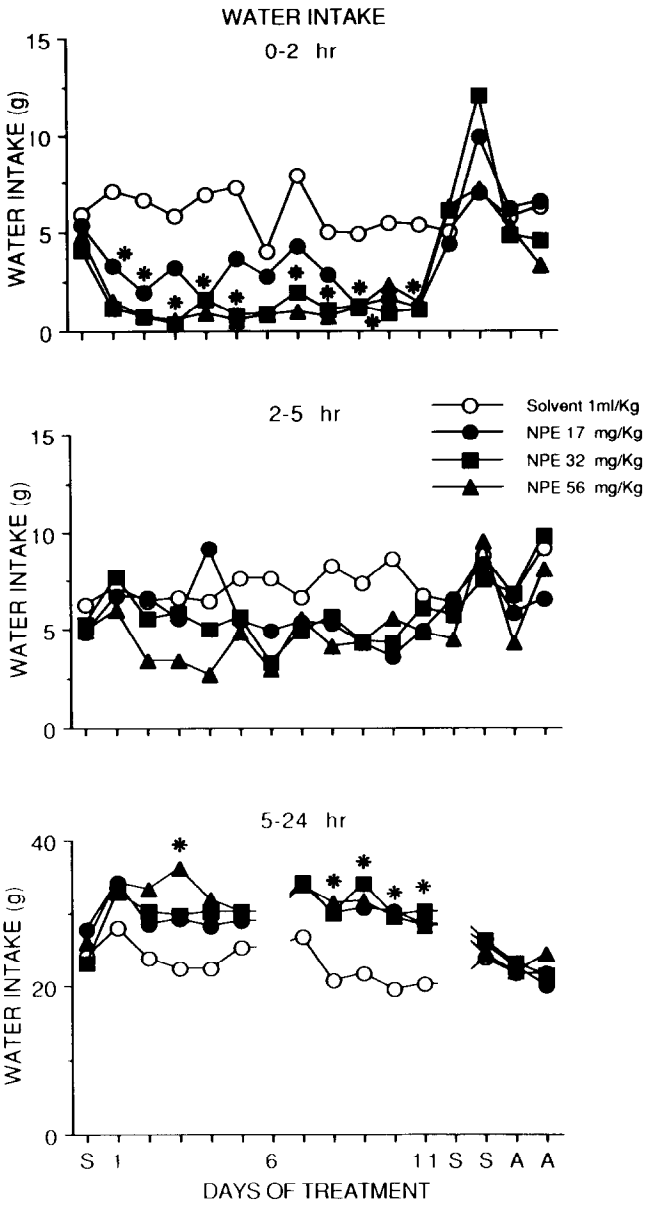

FIG. 2. Mean fractional water intake at 2, 5, and 24 h in groups treated with solvent or with the three doses of NPE. Same symbols as in Fig. 1. $*p < 0.05$ vs. solvent group; Tukey's test.

18) = 0.375, p = 0.772, and within subjects, $F(30, 180)$ = 0.738, p = 0.836) (data not shown).

Urine Output

On the first day of treatment NPE significantly increased urine output at 2 h only. The lowest dose (17 mg/kg) of the drug already yielded the maximal diuretic effect (Fig. 4). However, the diuretic effect of NPE gradually disappeared and from the second day on ANOVA disclosed no overall drug effect, $F(3, 18)$ = 1.919, p = 0.163).

DISCUSSION

The present study confirms and extends the notion that NPE inhibits ingestive behavior in rats. It shows that the suppressant effect of NPE is short lasting and affects food intake more than water intake. In addition, we observed that the anorectic effect of NPE is not modified by 11 days of treatment; the discontinuation of NPE administration does not lead to an overshoot of food and water intake; and NPE treatment does not alter the ingestive response to amphetamine. As already mentioned, under free-feeding conditions daily treatment with amphetamine progressively activates drinking and feeding between 2 and 5 h after drug administration (2,10,14). This activation usually results in a remarkable hyperdipsic response and in a normalization of food intake at 5 h. Upon discontinuation of amphetamine treatment food intake overshoots, whereas water intake tends to normalize. Thus, the suppressant effects of NPE on ingestive behavior seem to differ remarkably from those of amphetamine. In particular, NPE seems to lack the stimulant action on ingestive behavior, typically shown by amphetamine (4) and emphasized by its chronic administration. Pharmacological differences between NPE and amphetamine are further supported by the way the 2 drugs affected urine output: NPE induced a weak diuretic effect that disappeared after the first day of

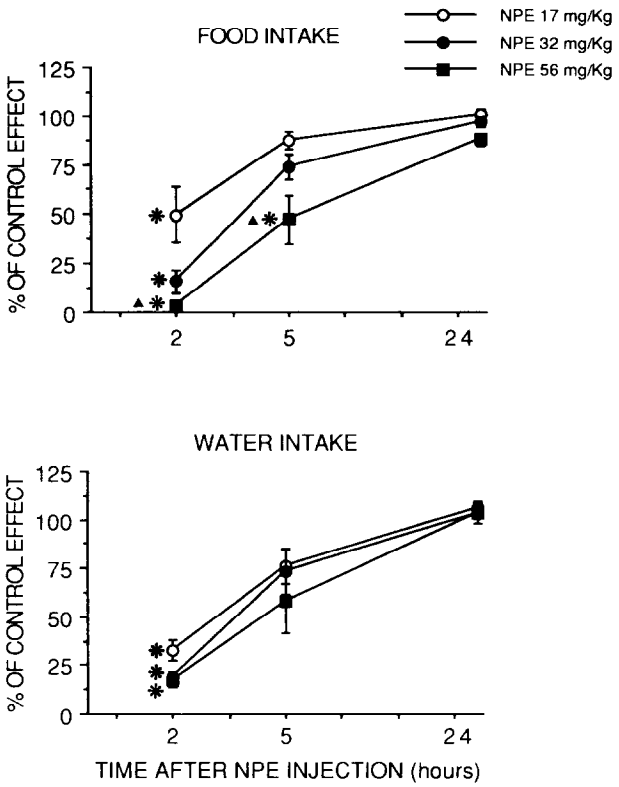

FIG. 3. Cumulative food (upper panel), or water intake (lower panel), at 2, 5, and 24 h was averaged across the 11 days of NPE treatment. The effect of NPE is shown as percent of the control response. *p < 0.05 vs. solvent group; Δp < 0.05 vs. NPE17 mg/kg; Tukey's test.

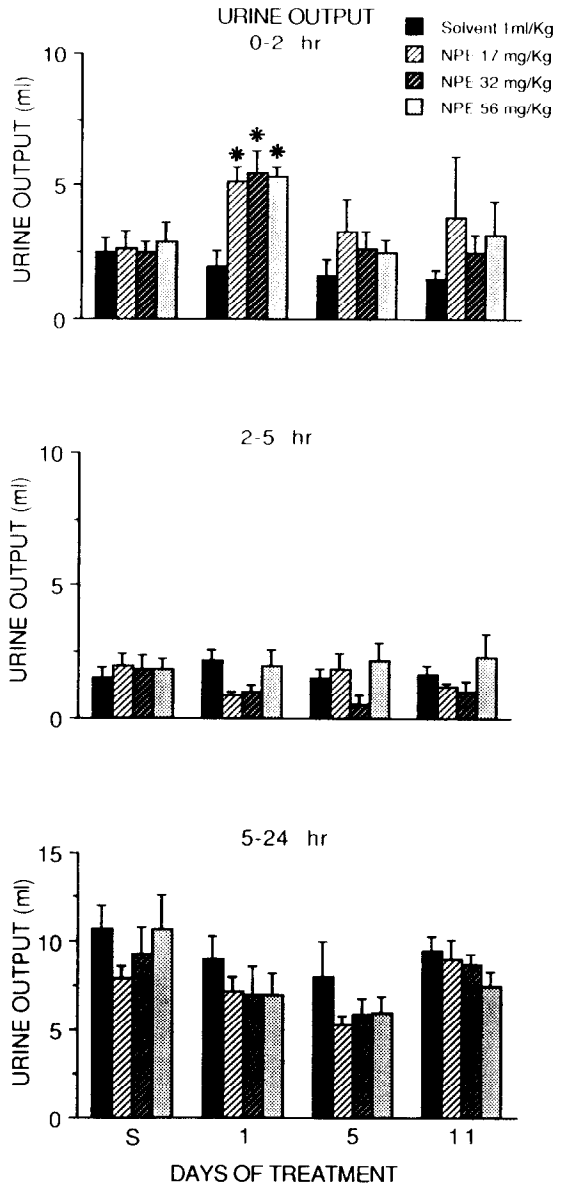

FIG. 4. Mean fractional urine output at 2, 5, and 24 h in groups treated with solvent or with the three doses of NPE. *p < 0.05 vs. solvent group; Tukey's test.

treatment, whereas amphetamine-induced diuresis remained stable throughout weeks of treatment (10).

In an attempt to assess the amphetamine-like behavioral effects of NPE, in a water-reinforced fixed-ratio 20 two-lever operant behavior (20 responses on the appropriate lever gave access to the reinforce), we trained rats to discriminate NPE 30 mg/kg IP from solvent (unpublished results). Only 4 of the 10 trained rats met discrimination criteria (80% of the responses emitted on the correct lever before the first reinforcement in 7 out 8 days of training) and 2 of them generalized amphetamine (at doses of 0.56 and 1.0 mg/kg IP) for NPE. Note, however, that after an average of 30 training sessions the four rats lost their ability to discriminate NPE. This finding suggests that at fully anorectic doses, NPE has very weak discriminative stimulus properties, which are only marginally of amphetamine-like type. Thus, NPE remarkably differs from its stereoisomer cathine, which fully generalizes for amphetamine in a drug discrimination paradigm (16). Because drugs that share discriminative stimulus properties usually also share reinforcing properties, we may argue that at the best NPE has weak amphetamine-like addictive properties.

In conclusion, the pharmacological effects of NPE seem to differ substantially from those of amphetamine. Pharmacokinetic reasons are unlikely to account for these differences. We have no information about the distribution of NPE in the body, but it is unlikely that it differs substantially from that of its enantiomers phenylpropanolamine and cathine. These compounds are less lypophilic than amphetamine, yet they cross the blood-brain barrier in a sufficient amount to produce central pharmacological effects (7,17). In particular, the anorectic effect of phenylpropanolamine is mainly attributed to its ability to activate alpha-1 adrenergic receptors within the hypothalamic paraventricular nucleus (19). The observation that in our study the highest NPE doses (32 and 56 mg/kg) produced some degree of motor activation (locomotion and head bobbing) supports the view that the drug has central effects. Nevertheless, in producing its suppressant effects NPE may also act peripherally, as do phenylpropanolamine and even by amphetamine (6,18). Among the several peripheral mechanisms that could lead to the inhibition of the ingestive behavior, particularly worth testing is the possibility that NPE produces taste aversion, as sympathomimetics usually do (6).

Yet another reason makes a more detailed study of the suppressant effect of NPE on ingestive behavior interesting. Not only is this compound one of the two major products of the metabolism of cathinone, the most potent active principle of *Catha edulis* (khat) in humans, but a recent study has shown that plasma concentrations of NPE remarkably outlast those of cathinone (20). This raises the question as to whether the persistent presence of this metabolite in the organism has pharmacological consequences. If so, NPE may contribute substantially to the well-known anorectic effect of khat chewing, so far exclusively referred to cathinone and cathine adsorption (7,11).

Considering this compound in a therapeutic perspective, because NPE lacks an effect on body weight it is unlikely to be useful for weight control in humans. Its short-lasting suppressant action on ingestive behavior and its apparently tolerance-free anorectic properties may, however, allow this drug to be used as an adjuvant in the therapy of eating disorders characterized by binge episodes.

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