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Pharmacological Basis for N-*n*-Propylnorapomorphine Induced Stereotypic Cage Climbing and Behavioral Arousal in Mice

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WILCOX, R. E., W. H. RIFFEE AND R. V. SMITH. *Pharmacological basis for N-n*-propylnorapomorphine induced stereotypic cage climbing and behavioral arousal in mice. PHARMAC. BIOCHEM. BEHAV. 11(6) 653–659, 1979.—Apmorphine (APO) and its N-propyl homologue (N-*n*-propylnorapomorphine; NPA) are approximately equipotent in inducing stereotypic cage climbing and behavioral arousal. The time courses for the two behavioral responses of both aporphines are also quite similar. These results suggested that aporphine-induced stereotypic cage-climbing and behavioral arousal, if specific for dopamine receptor stimulation, could provide useful *in vivo* models for predicting dose- and time-response effects of potential antiparkinsonian agents. In the present experiments, six neurotransmitter receptor blockers (atropine, phentolamine, sotalol, cyproheptadine, naloxone, and haloperidol or spiroperidol) were compared in mice for their ability to alter cage climbing and behavioral arousal induced by NPA. Results indicated that pretreatment with the dopamine blockers haloperidol and spiroperidol, significantly antagonized both responses to NPA and shifted the cage climb dose response curve to the right 15 - fold. In contrast, the muscarinic cholinergic (atropine), alpha-noradrenergic (phentolamine), beta-noradrenergic (sotalol), serotonergic (cyproheptadine), and opiate (naloxone) receptor blockers uniformly did not attenuate activity due to NPA. These results suggest that cage-climbing and arousal induced by aporphines is mediated via dopamine receptor stimulation and that these responses provide useful in vivo models for accurate evaluation of certain classes of dopamine agonists with clinical utility.

Parkinson's disease Aporphines Receptor blockers Stereotypy Arousal

INTEREST in apomorphine (APO) and its analogs has been renewed recently because of its documented antiparkinsonian effects [1] either alone or supplemental to L-dopa [2,3]. Also, APO has been suggested as a potentially useful antidyskinetic [5,7] and antipsychotic [16] agent. Furthermore, the dopaminergic properties of APO make it an important tool in biochemical investigations of dopamine receptor function [13]. Recent work has suggested that the N-*n*-propyl homologue of APO (N-*n*-propylnorapomorphine; NPA) offers considerable promise as a therapeutic agent for treating Parkinsonism [4,17] possibly because of its greater *in vivo* potency [8,11] in some test systems. In previous research, we evaluated the potencies and time courses for APO and NPA in three model systems in mice: stereotypic cage-climbing, hypothermia [11], and behavioral arousal ([10] Wilcox *et al.*, submitted-a). The time courses of action for APO and NPA were found to closely parallel each other and blood APO levels [14] for stereotypy and arousal but not hypothermia. The potencies of the two aporphines were found to be quite similar for the cage-climb and arousal responses [10,11] but markedly divergent for hypothermia (NPA approximately 100 times as potent as APO; [11]. The hypothermic response induced by both aporphines is due to dopamine receptor stimulation (Wilcox *et al.*, submitted-b) yet hypothermia, for lack of a possible relationship with blood levels, appears to offer less promise as an animal model for predicting clinically useful aporphines in Parkinson's disease. The present investigation attempts to determine the pharmacological basis for the cage-climbing and arousal responses induced by NPA.

METHOD

Subjects

Experimentally naive CD-1 male albino mice (Charles River) weighing between 20 and 36 g at the time of testing were used in all investigations. The animals were permitted access to food and water ad lib. Animals were maintained on a 12 hr light-dark cycle (lights on 6 a.m. and 6 p.m.) and all pharmacological testing was carried out between 9 a.m. and 5 p.m.

Experimental Design

The evaluation of stereotypic cage climbing consisted of two between subjects factors, BLOCKER (pretreatment;

saline, spiroperidol, atropine, phentolamine, sotalol, cyproheptadine, or naloxone) and DOSE (of NPA) and one within subjects factor CAGE CLIMB SCORE (cage climb scores each 5 min for 1 hr after drug administration).

The evaluation of behavioral arousal consisted of two between subjects factors, BLOCKER (pretreatment; saline, haloperidol, atropine, phentolamine, sotalol, cyproheptadine, or naloxone) and DRUG (APO or NPA) and one within subjects factor, BEHAVIORAL AROUSAL SCORE (5 min activity counts for 2 hr after drug administration). Statistical analyses of the data were carried out by appropriate use of t-tests, analyses of variance (ANOVA), and post-hoc multiple comparison procedures as previously described [19].

Drugs

Drugs used for the experiments were R-(-)-apomorphine hydrochloride hemihydrate (MacFarland Smith, Ltd., Edinburgh, Scotland), N-*n*-propylnorapomorphine hydrochloride (Sterling-Winthrop, Rensselaer, NY), phentolamine hydrochloride (CIBA, Summit, NJ), (-)-sotalol hydrochloride (Mead Johnson, Evansville, IN), atropine sulfate (City Chemical, New York, NY), cyproheptadine hydrochloride (Merck Sharp and Dohme, Rahway, NJ), naloxone hydrochloride (Endo Laboratories, Garden City, NY), haloperidol and spiroperidol (Janssen Pharmaceuticals, New Brunswick, NJ). Drugs were freshly prepared in distilled water without preservatives. Halperidol and spiroperidol were prepared in distilled water containing tartaric acid (1 mg butyrophenone / 1 mg tartaric acid). Isotonic saline was used as a control solution. Doses of the antagonists were selected in accord with previous research which indicated blockade of the appropriate receptor system with minimal behavioral responses; atropine=0.5 mg/kg; phentolamine=5.0 mg/kg; (-)-sotalol=10.0 mg/kg; cyproheptadine=0.1 mg/kg; naloxone=5.0 mg/kg; haloperidol=1.0 mg/kg; and spiroperidol=0.2 mg/kg; (all drugs administered IP; Wilcox *et al.*, submitted-a). In the cage-climb experiments dose-response analyses were carried out from 0.5–100 mg/kg NPA. In the arousal experiments doses of the aporphine (5 mg/kg, IP) were selected to provide a high baseline activity that would be susceptible to antagonism by receptor blockers [10].

Cage Climbing Behavior

A modification of the basic procedure of Protais [9] was employed throughout (involving videotaping of behavior coupled with blind ratings [9,20]). Briefly, animals were given one of the pretreatments indicated above (which acts additionally to minimize nonspecific effects of the handling/injection routine [12] and placed individually into cylindrical cages, 12 cm diameter, 14 cm high, with walls of verticle bars, 2 mm diameter, 1 cm apart, surmounted by fine wire mesh. Following a 60 min habituation period animals were given a pre-NPA rating of their behavior (see below), administered a dose of NPA and the behavior of the animals recorded on videotape (30 sec every 5 min) for the next hour. Videotaped behavior, scored *via* a 0–2 rating scale [9]: 0=four paws on cage floor; 1=two paws holding the verticle bars of the cage; 2=four paws holding the verticle bars of the cage, was later rated "blind" using procedures previously described [20]. Because of the short duration of action of naloxone, subjects in the naloxone/NPA groups received a saline preinjection followed 60 min later by simultaneous injections of naloxone (5 mg/kg) and NPA (as above).

Behavioral Arousal

Experimental and control animals received identical preinjections of one of the 6 blockers or isotonic saline (IP) 30 min prior to behavioral testing. Home cages (containing one mouse each) were placed in a testing room adjoining the colony room to habituate to apparatus noise. (In previous research not yet published, we compared the dose- and time-response effects of APO, NPA, dextroamphetamine, and levoamphetamine in animals run 3 to a cage vs 1 to a cage and found that responses are essentially identical, [15] Wilcox et al., submitted-c). The preinjection/homecage/habituation procedure minimizes artifactual increments in arousal due to exploratory activity and stress from the handling/injection routine [12]. At the time of testing, the control animal was administered isotonic saline and the experimental animal was given 5 mg/kg (IP) of APO or NPA. The two home cages were placed on electromagnetic sensing stages (Stoelting Co., model 31401, sensitivity=0.7 mA) for monitoring of changes in behavioral arousal in simultaneously run drug-injected and control animals. Five min activity counts were recorded automatically for 90 min commencing immediately after drug/saline injection. As discussed in previous reports [12] behavioral arousal in the present context refers to a composite behavior which includes locomoter activity, rearing, and repetitive movements.

RESULTS

Figure 1 presents dose-response analyses of cage-climbing to NPA after various pretreatments. In Figure 1A are shown the effects of a saline pretreatment on cage climbing induced by NPA given over a dose range approximately one order of magnitude greater than the threshold dose of NPA required to produce stereotypic activity [11]. The ED50 dose of NPA in the cage-climb experiments was defined as that dose which produced a cage-climb score equal to 50% of that induced by 5 mg/kg NPA (i.e. a score of 4.8 out of a theoretical maximum of 24). The ED50 for saline/NPA is 2.0 mg/kg (Figure 1A, Table 1). The effect of spiroperidol (0.2 mg/kg) on cage climbing induced by NPA is presented in Fig. 1B. Pretreatment with the butyrophenone shifts the NPA dose-response curve to the right by an order of magnitude with an ED50 calculated to be approximately 30 mg/kg (Table 1). Pretreatment with atropine (Fig. 1C), phentolamine (Fig. 1D), (-)-sotalol (Figure 1E), cyproheptadine (Fig. 1F), or naloxone (Fig. 1G) resulted in minimal effects on the cage-climbing induced by NPA. In Table 1 are presented the ED50's for each pretreatment/NPA combination and the potency ratios determined by comparing the NPA ED50 for each blocker with that for saline/NPA. The potency of NPA in inducing stereotypic activity in the presence of all non-dopaminergic antagonists is essentially the same as that in the presence of saline whereas sprioperidol pretreatment necessitates a 15-fold increase in the amount of NPA required to produce a half-maximal response.

Figure 2 presents time-response curves for saline/APO and saline/NPA effects on behavioral arousal. Periods in which arousal following the aporphine was significantly greater than activity following saline are indicated by as-

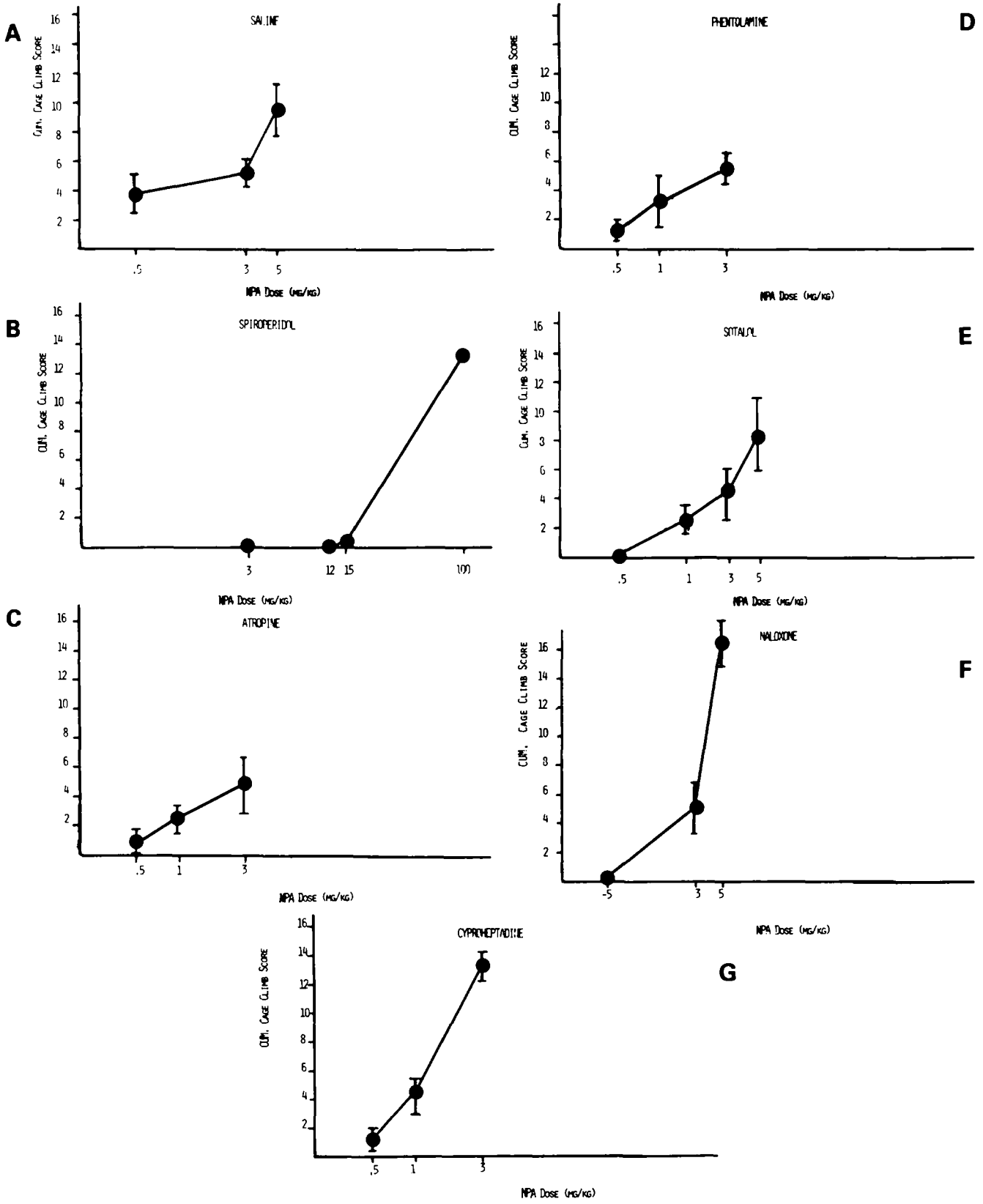

FIG. 1. Dose-response curves for stereotypic cage climbing induced by N-*n*-proplynorapomorphine (NPA) following pretreatment with various drugs. Stereotypic activity was expressed as mean ± SEM cumulative stereotypic acitivity for the 60 min immediately following NPA. (n=at least 6 mice per group). Pretreatments: isotonic saline (1A), spiroperidol (1B), atropine (1C), phentolamine (1D), sotalol (1E), cyproheptadine (1F), or naloxone (1G). Refer to text for details of methods.

TABLE 1

EFFECT OF NEUROTRANSMITTER ANTAGONISTS ON STEREOTYPIC CAGE CLIMBING TO N-*n*-PROPYLNORAPOMORPHINE

Pretreatment (mg/kg)	NPA ED50 (mg/kg)*	Potency† ratio
A Saline	2.0	—
B Spiroperidol (0.2)	30.0	15
C Atropine (0.5)	3.0	1.5
D Phentolamine (5.0)	2.3	1.1
E (−)-Sotalol (10.0)	3.3	1.6
F Cyproheptadine (0.1)	2.8	1.4
G Naloxone (5.0)	1.1	0.6

*ED50s were calculated by dropping perpendiculars from the log dose-response curves from the 1/2 maximal cage climb score of 4.8.

†Potency ratios were calculated by comparing NPA ED50s for each blocker with that for saline/NPA.

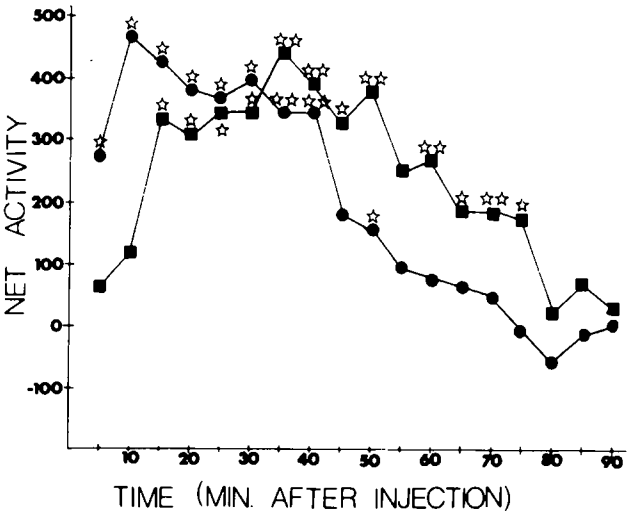

FIG. 2. Time-response curves for behavioral arousal induced by apomorphine (APO) and N-*n*-propylnorapomorphine (NPA). Arousal was expressed as mean activity counts of drug injected animals minus that of saline injected animals run for consecutive 5 min periods beginning immediately after drug injection. Both drug and saline-injected animals received a saline preinjection 60 min prior to injection of drug or saline to minimize extraneous effects of handling/injection procedure (see text). N ≥ 6 mice per group. APO = ●; NPA = ■. Drug vs saline: ☆ = $p<0.05$, ☆☆ = $p<0.01$.

terisks ($p<0.05$). Saline/APO produced a peak response (R_{max}) of 468 activity counts at 10 min (t_{max}), with 9 periods of significantly enhanced arousal. Saline/NPA similarly resulted in an $R_{max}=437$ counts at 35 min with 11 periods of significant arousal.

Figure 3 presents time-response curves for the six blocker/aporphine combinations. In contrast to the results obtained with other receptor blockers, haloperidol/APO resulted in essentially no net arousal (relative to haloperidol/saline) throughout the testing session (Fig. 3A). Furthermore, haloperidol/saline significantly depressed activity at 7 times from 5–50 min relative to saline/APO. Similarly, haloperidol/NPA resulted in essentially no net arousal relative to haloperidol/saline. Relative to saline/NPA, haloperidol/NPA animals manifested 7 periods of significantly depressed activity. Thus, haloperidol antagonized the arousal response to both APO and NPA.

Following atropine/APO (Fig. 3B), a maximum response occurred at 35 min (370 counts) with significant arousal from 20–40 min. Comparison between atropine/APO and saline/APO groups at each time period indicated no significant differences. Thus, atropine did not affect the behavioral arousal induced by APO. The R_{max} to atropine/NPA (213 counts) occurred at 50 min with 7 periods in which significant arousal occurred. Essentially no differences were observed between atropine/NPA and saline/NPA animals (2 periods out of 18 in which an atropine-induced depression of activity occurred relative to saline/NPA).

Phentolamine/APO produced a maximal arousal response of 473 counts at 15 min and 8 periods of significant activity during the monitored 90 min (Fig. 3C). Comparisons with saline/APO animals indicated no 5 min periods in which net activity differed between the two groups. Thus, phentolamine failed to significantly modify the activity response to APO. Phentolamine/NPA resulted in an $R_{max}=509$ counts at 25 min with a significant enhancement of arousal occurring for 10 periods. There were no differences in activity between the phentolamine/NPA and saline/NPA mice.

Sotalol/APO (Fig. 3D) induced a maximal response at 50 min (316 counts) with significant activity from 35–50 min. Relative to saline/APO essentially no significant differences were observed. Thus, sotalol did not antagonize the arousal effect of APO. The maximal response to sotalol/NPA was obtained at 40 min (225) counts with significant activity from 35 to 40 min. With the exception of two 5 min periods, sotalol/NPA animals showed arousal responses indistinguishable from those of saline/NPA mice.

Cyproheptadine/APO produced an R_{max} of 371 counts at 25 min with 10 significant activity periods during the session (Fig 3E). Essentially no differences between activity induced in cyproheptadine/APO and saline/APO animals were observed. In contrast, cyproheptadine/NPA produced significant activity throughout the monitored periods (18 out of 18 significant periods). However, relative to saline/NPA, cyproheptadine/NPA showed similar arousal throughout the 90 min period.

Naloxone/APO (Fig 3F) resulted in maximal responding at 30 min ($R_{max}=559$ counts) with significant activity from 30–45 min but no differences from saline/APO throughout the 90 min. Naloxone/NPA similarly produced significant arousal from 15–55 min but also no differences relative to saline/NPA. A second separate experiment (not shown) was carried out in which experimental and control mice received a saline preinjection followed by injections of naloxone/saline or naloxone/NPA. Naloxone/NPA and saline/NPA produced similar effects on arousal under these conditions also.

Table 2 presents comparisons between APO vs NPA mice for each of the seven pretreatment combinations used (saline, haloperidol, atropine, phentolamine, sotalol, cyproheptadine, and naloxone). Essentially no differences were found in the effects of any pretreatment on APO vs NPA behavioral arousal throughout the 90 min following injection.

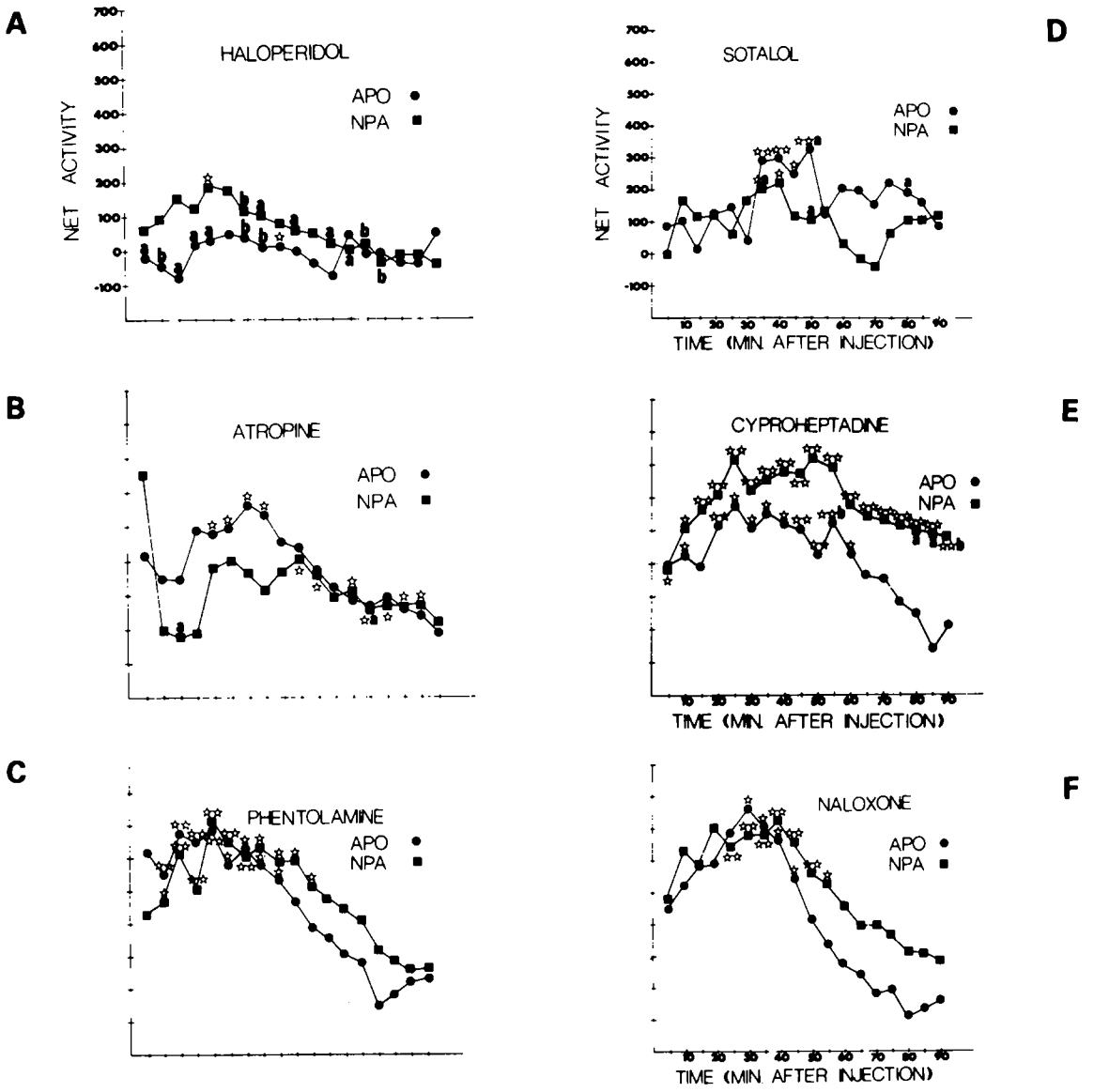

FIG. 3. Time-response curves for behavioral arousal induced by apomorphine (APO) and N-*n*-propylnorapomorphine (NPA) following pretreatment with receptor blockers. Arousal was expressed as mean activity counts of blocker/saline animals run for consecutive 5 min periods beginning immediately after drug injection. n ≥ 6 mice per group. Blocker/APO=●; Blocker/NPA=■. Blocker key: Haloperidol=upper left; Atropine=middle left; Phentolamine=bottom left; (-)-Sotalol=upper right; Cyproheptadine=middle right; Naloxone=bottom right. Blocker/drug vs Blocker/saline: ☆=$p<0.05$, ☆☆=$p<0.01$.

DISCUSSION

Sterotypic cage climbing and hypothermia in mice have been proposed as models of specific dopaminergic activity for apomorphine [6,9]. We have recently reported dose- and time-response comparisons of cage-climbing and hypothermia to APO and NPA. In these latter studies, it was shown that the potencies of APO and NPA differ dramatically in the two systems (cage climb ED_{50s}=1.95 and 3.3 mg/kg; hypothermia ED_{50s}=4.7 and 0.052 mg/kg respectively). The time courses of the two responses also differ with maximal cage climbing occurring from 5–30 min but maximal hypothermia from 30–60 min [18].

The cage-climb and hypothermic responses to APO have been substantiated as being mediated via dopamine receptor stimulation since haloperidol and pimozide, but not atropine, phentolamine, (±)-propranolol, (−)-sotalol, cyproheptadine, and naloxone antagonized both responses [18]. Furthermore, similar effects were found with the hypothermic response elicited by NPA. In the present investigation of the dopaminergic, adrenergic, cholinergic, serotonergic and narcotic receptor blockers, only haloperidol antagonized the arousal response to APO and NPA and only spiroperidol shifted the NPA-induced cage-climb response curve to the right.

It should be noted that the aporphine-induced increases in arousal, which appear to correlate with plasma drug levels

TABLE 2

COMPARISONS OF BEHAVIORAL AROUSAL IN APOMORPHINE- vs. N-*n*-PROPYLNORAPOMORPHINE-TREATED MICE FOLLOWING PRETREATMENT WITH NEUROTRANSMITTER BLOCKERS OR SALINE

Time after injection	Saline	Atropine	Phentolamine	Sotalol	Naloxone	Cyproheptadine	Haloperidol
5	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—
15	—	—	—	—	—	—	A<N*
20	—	—	—	—	—	—	—
25	—	—	—	—	—	—	A<N*
30	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—
45	—	—	—	—	—	—	—
50	A<N*	—	—	N<A†	—	A<N*	—
55	—	—	—	—	—	A<N*	—
60	A<N*	—	—	—	—	—	—
65	—	—	—	—	—	—	—
70	—	—	—	—	—	—	—
75	—	—	—	—	—	A<N*	—
80	—	—	—	—	—	A<N*	—
85	—	—	—	—	A<N*	A<N*	—
90	—	—	—	—	—	A<N*	—

Column heading represent pretreatments prior to apomorphine (APO) or N-*n*-propylnorapomorphine (NPA). Entries constitute periods in which arousal significantly differed in APO and NPA injected animals following pretreatment with a blocker.

A=APO; N=NPA; *$p<0.05$; †$p<0.01$.

(unpublished data) are manifest only in animals habituated both to the testing environment (via testing in the "home cage") and to the handling/injection routine [12]. The present results may be due in part to the elimination of portions of the exploratory or stress-induced arousal from the monitored responses which account for the mixed results reported in the apomorphine arousal literature (see [1] for review).

We have previously shown that APO and NPA have similar potencies and time courses in inducing sterotypic cage-climbing and behavioral arousal in mice. Our previous results with hypothermia, ([18]; Wilcox *et al.*, submitted-a) are entirely consistent with the present data which indicated specific blockade of the aporphine-induced responses only by dopamine antagonists. These results suggest that aporphine-induced behavioral arousal and cage climbing in mice could provide useful *in vivo* tests for predicting dose- and time-response effects of potential therapeutic antiparkinsonian drugs.

ACKNOWLEDGEMENTS

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