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Differentiation of Neuropharmacological Actions of Apomorphine and d-Amphetamine[1]

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QUOCK, R. M. AND A. HORITA. *Differentiation of neuropharmacological actions of apomorphine and d-amphetamine.* PHARMAC. BIOCHEM. BEHAV. 5(6) 627–631, 1976. – The dopaminergic agonists apomorphine and d-amphetamine elicit hyperthermic, hyperkinetic and stereotypic responses in the rabbit. The present investigation compares the influence exerted by various serotonergic antagonists upon these activities. Apomorphine-induced hyperthermia was antagonized by p-chlorophenylalanine (pCPA), cyproheptadine and cinanserin and was restored in pCPA-pretreated rabbits by regeneration of central serotonin levels. d-Amphetamine-induced hyperthermia was reduced by pCPA; restored in pCPA-pretreated animals by regeneration of central serotonin levels; and was uninfluenced by cyproheptadine and cinanserin. Apomorphine-induced locomotor stimulation was unaltered by serotonergic antagonists; however, these same doses of anti-serotonergic agents all markedly reduced d-amphetamine-induced hyperkinesia. Serotonergic antagonists also failed to affect apomorphine-induced compulsive gnawing but did significantly enhance d-amphetamine-induced compulsive gnawing. It is concluded from these data that the neuropharmacological activities of apomorphine and d-amphetamine in the rabbit differ in their dependence upon central serotonergic mechanisms.

Apomorphine d-Amphetamine Locomotor activity Body temperature Stereotyped behavior Serotonin

APOMORPHINE and d-amphetamine have been widely utilized as investigative tools in research on central monoaminergic mechanisms. The neuropharmacological actions of these two agents have been attributed to a common mechanism: the stimulation of central dopaminergic receptors. It was previously thought that the only distinction between these dopaminergic agonists was that apomorphine acted directly upon dopamine-sensitive receptors, whereas d-amphetamine released transmitter from dopamine-containing nerve terminals to stimulate an adjacent dopaminergic receptor [4,5].

We have conducted extensive studies using apomorphine and d-amphetamine as dopaminergic agonists in the rabbit. The syndrome induced in the rabbit by apomorphine and d-amphetamine includes: (a) an increase in the colonic temperature; (b) increased locomotor activity; and (c) stereotyped behavior in the form of compulsive gnawing [11, 12, 13]. These drug effects are antagonized by dopaminergic receptor blockers and can be evoked by intraventricular administration of either agonist [11,18].

In recent years, the question of whether the indoleamine serotonin (5-hydroxytryptamine) might participate in mediation of some of the central effects of apomorphine or d-amphetamine has been raised. Experiments in rodents have yielded conflicting findings [2, 3, 6, 14, 23]. An earlier study by us suggested that apomorphine-induced hyperthermia but not locomotor stimulation or compulsive gnawing in the rabbit might be dependent upon central serotonergic mechanisms [19]. If d-amphetamine, the indirect-acting counterpart of apomorphine, activates the same dopaminergic mechanisms that are directly stimulated by apomorphine, then serotonergic antagonists would be expected to similarly interfere with the hyperthermic but not the hyperkinetic or stereotypic actions of d-amphetamine in the rabbit. The present investigation is an extension of our earlier research and compares the extent of serotonergic involvement in the neuropharmacological activities of apomorphine and d-amphetamine in the rabbit.

METHOD

Animals

Male New Zealand rabbits (1.8–2.3 kg, Totem Farms, Washington) were restrained in open wooden stanchions, as described by Shellenberger and Elder [21], for 6–8 hr on the day prior to experimentation. Drinking water was freely available and the room temperature was regulated at 22.0° ± 1.0° C.

Procedure

Determination of colonic temperature. The colonic temperatures of all animals were electronically monitored and recorded with the use of flexible rectal thermistor probes connected to an automatic telethermometer (Yellow Springs Instruments) and a multiple-channel recorder

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(Leeds and Northrup). The significance of any observed differences in colonic temperature responses between control and experimental groups was determined at 15-min intervals after injection, using Student's *t*-test.

Quantitative assessment of locomotor activity. A subjective rating scale was developed for the purpose of quantifying drug-induced changes in locomotor activity: 0 points, rabbit is lying down and inactive; 1 point, rabbit is crouching on all fours and quiescent; 2 points, rabbit is crouching on all fours but is alert and restless; 3 points, rabbit is standing on all fours and demonstrates mild or sporadic locomotor activity; and 4 points, rabbit is standing on all fours and engaged in intense or continual locomotor activity. A locomotor activity index was assigned to each animal at 15-min intervals after injection and the significance of any differences in locomotor response between control and experimental groups was determined, using Student's *t*-test.

Determination of stereotyped behavior (compulsive gnawing). Rabbits were observed for any demonstration of compulsive gnawing following drug administration and the incidence of stereotypy occuring within 60 min of injection was recorded for each group of animals. Compulsive gnawing was judged to be present as the animal gnawed continually for 30 sec and after its head was gently turned aside to interrupt this behavior, the animal immediately resumed gnawing. The significance of any observed differences in the incidence of gnawing between control and experimental groups was determined, using the Chi-squared (2×2 contingency table) test [8].

Drugs. The following drugs were used in this study: apomorphine hydrochloride (Merck Sharp and Dohme); d-amphetamine sulfate (Sigma); p-chlorophenylalanine methyl ester hydrochloride (pCPA) (Nutritional Biochemicals); cyproheptadine (Merck Sharp and Dohme); cinanserin (Squibb); 1-β-(3,4,-dihydroxyphenyl-α-hydrazino-α-methylpropionic acid (Carbidopa) (Merck Sharp and Dohme); and DL-5-hydroxytryptophan (5-HTP) (Regis). All drug solutions were prepared in saline.

Determination of rabbit brain serotonin content. Control and drug-pretreated rabbits were sacrificed by air embolism and the brains were removed from the calvarium. The cerebral cortex and cerebella were discarded and the remaining brainstem regions were homogenized in dilute hydrochloric acid and assayed for serotonin, using the method of Bogdanski *et al.* [1]. The brainstem serotonin contents of control and various experimental groups were compared and the significance of any differences were determined, using Student's *t*-test.

RESULTS

Effects of Dopaminergic Agonists in Control Rabbits

The intravenous administration of apomorphine, 4.0 mg/kg (as the base), and d-amphetamine, 5.0 mg/kg (as the base), induced mydriasis and increased the rate of respiration in naive rabbits. Changes in the vasomotor state of the ears were also evident, apomorphine producing vasodilation and d-amphetamine exerting a vasoconstrictory effect. Both drugs also produced compulsive gnawing; however, this was not a consistent response in all animals at these doses. Eight of 28 (29%) rabbits receiving apomorphine exhibited this response, while in another group of naive animals, d-amphetamine produced compulsive gnawing in 6 of 26 (23%) rabbits.

Both apomorphine and d-amphetamine markedly elevated colonic temperature. Apomorphine produced a maximum temperature change of +1.5°C 45–60 min after injection, while the d-amphetamine-induced hyperthermia peaked at +2.2°C 60 min after drug administration. The duration of each hyperthermic response was about 3 hr (Fig. 1). Both apomorphine and d-amphetamine also produced an increase in locomotor activity, the height of locomotor stimulation generally occurring within 15 min of injection. The mean locomotor activity index for apomorphine-treated rabbits at this time was 3.4, while those treated with d-amphetamine peaked at 2.8. The locomotor stimulation was usually followed by a period of compensatory hypoactivity lasting approximately 60 min (Fig. 2).

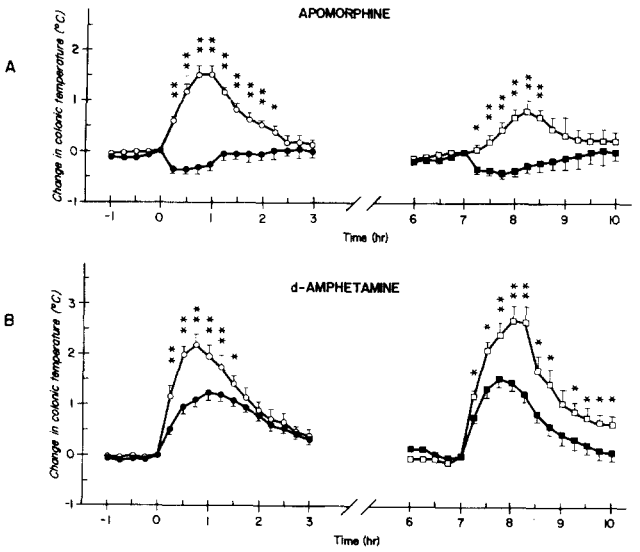

FIG. 1. Temperature effects of dopaminergic agonists in serotonin-depleted and serotonin-restored rabbits. A: ○, apomorphine (4.0 mg/kg); ●, pCPA (3 × 300 mg/kg) + apomorphine (4.0 mg/kg); □, Carbidopa (25 mg/kg) + 5-HTP (10 mg/kg) + apomorphine (4.0 mg/kg); and ■, saline + apomorphine (4.0 mg/kg). B: ○, d-amphetamine (5.0 mg/kg); ●, pCPA (3 × 300 mg/kg) + d-amphetamine (5.0 mg/kg); □, Carbidopa (25 mg/kg) + 5-HTP (10 mg/kg) + d-amphetamine (5.0 mg/kg); and ■, saline + d-amphetamine (5.0 mg/kg). All curves represent the mean temperatures of at least six rabbits; pCPA pretreatment group consisted of 12 animals subsequently divided into two groups of 6 each. Vertical lines represent SEM. Significance of difference: $*p<0.05$; $**p<0.01$.

Effects of Dopaminergic Agonists in Rabbits Pretreated with pCPA

Colonic temperature was not significantly influenced by pretreatment with pCPA, 300 mg/kg, IP, 3 times over a 48-hr period. When such pCPA-pretreated animals were administered apomorphine, the hyperthermia was abolished and the hypothermic component of apomorphine action was observed. These animals were then randomly divided into 2 equal groups. One group was treated with Carbidopa, 25 mg/kg, IP; after 60 min, 5-HTP, 10 mg/kg, was intravenously administered. This injection of 5-HTP produced an immediate though significant fall in temperature of approximately 1 hr in duration. Three hours later, these rabbits were again normothermic and were challenged with

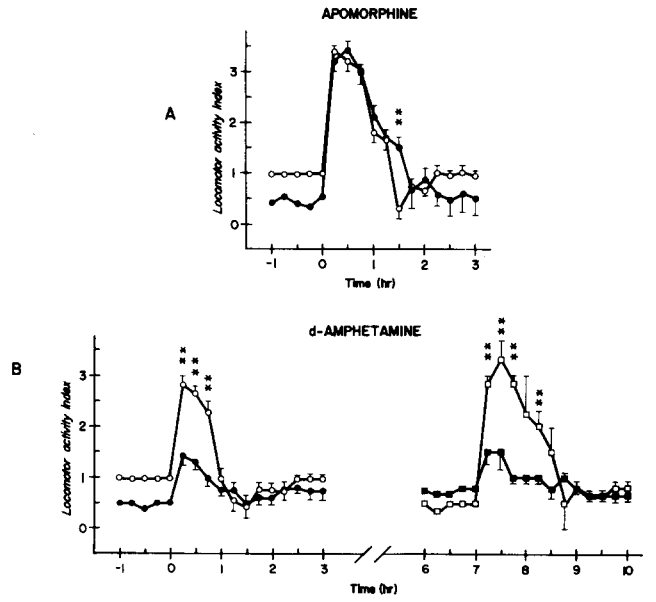

FIG. 2. Locomotor effects of dopaminergic agonists in serotonin-depleted and serotonin-restored rabbits. Same scheme as Fig. 1.

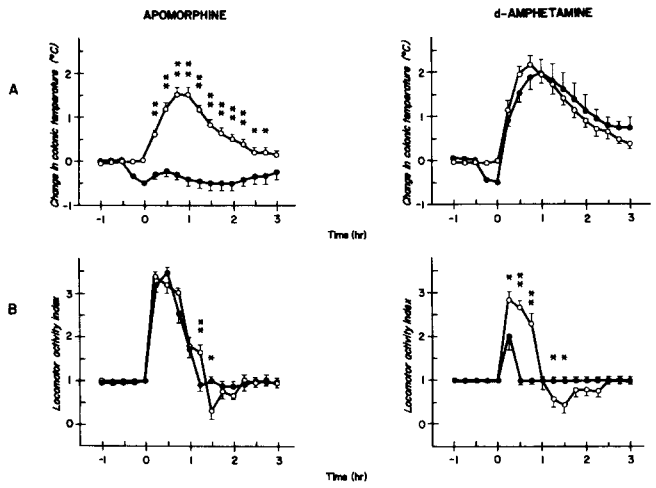

FIG. 3. Temperature and locomotor effects of dopaminergic agonists in cyproheptadine-pretreated rabbits. A: ○, apomorphine (4.0 mg/kg); ●, cyproheptadine (2.0 mg/kg) + apomorphine (4.0 mg/kg); ○, d-amphetamine (5.0 mg/kg); and ●, cyproheptadine (2.0 mg/kg) + d-amphetamine (5.0 mg/kg). B: Same scheme as Part A. All curves represent the mean temperatures or locomotor activity indices of at least six rabbits. Vertical lines represent SEM. Significance of difference: $*p<0.05$; $**p<0.01$.

a second injection of apomorphine. They responded with a significant rise in temperature. The other group of animals was administered injections of saline in lieu of Carbidopa and 5-HTP; these animals responded to the second apomorphine challenge with the same hypothermia seen earlier (Fig. 1A).

Pretreatment of rabbits with this same dose and regimen of pCPA also resulted in partial antagonism of the d-amphetamine-induced hyperthermia, the intensity of the temperature rise being reduced by about 50%. These pCPA-pretreated animals were also randomly divided into 2 equal groups. The first group received treatment with Carbidopa and 5-HTP, as described above. Then after a period of 3 hr, they were challenged with a second injection of d-amphetamine; these animals responded with a marked increase in temperature, the peak of which slightly exceeded the control response. The second group of rabbits received saline instead of Carbidopa and 5-HTP and continued to respond to d-amphetamine with the attenuated hyperthermia (Fig. 1B).

Intraperitoneal pretreatment with pCPA, 3 × 300 mg/kg, also induced a hypoactive state in naive rabbits; this was reflected as a 50% reduction in the locomotor activity indices. When these animals were treated with apomorphine, the typical locomotor stimulatory response was observed (Fig. 2A). This was in marked contrast to the results obtained when such pretreated rabbits were challenged with d-amphetamine. d-Amphetamine-induced locomotor hyperactivity was significantly reduced. In animals receiving injections of Carbidopa and 5-HTP, as described above, a hyperactive response to d-amphetamine was once again evident. The administration of saline instead of Carbidopa and 5-HTP had no such restorative effect on the reduced locomotor response to d-amphetamine in the second group of pCPA-pretreated rabbits (Fig. 2B).

Effects of Dopaminergic Agonists in Rabbits Pretreated with Serotonergic Receptor Blockers

Intravenous administration of cyproheptadine, 2.0 mg/kg, produced a slight reduction in rabbit colonic temperature; this 0.5° C fall in temperature lasted about 60 min. When these cyproheptadine-pretreated animals were challenged with apomorphine after 30 min, the hyperthermic response was markedly attenuated. On the other hand, cyproheptadine appeared not to influence d-amphetamine-induced hyperthermia (Fig. 3A). Though not illustrated, cinanserin, 10 mg/kg, IV, exerted similar influences upon the hyperthermic responses to apomorphine and d-amphetamine administered 30 min later.

While colonic temperature was slightly depressed by cyproheptadine, 2.0 mg/kg, IV, locomotor activity remained fairly normal, although the animals did appear to react more slowly to tactile stimuli. The administration of apomorphine to cyproheptadine-pretreated rabbits resulted in locomotor stimulation comparable to that seen in controls. However, the compensatory hypoactive component of the response to apomorphine was not observed. The d-amphetamine-induced locomotor stimulation was greatly reduced by cyproheptadine pretreatment, the initial intensity of the hyperkinesia being attenuated by about 50% and the duration of the response shortened to less than 30 min (Fig. 3B). Identical locomotor responses were obtained following pretreatment with cinanserin, 10 mg/kg, IV.

Influence of Various Drug Pretreatments on the Incidence of Stereotyped Behavior Induced by Dopaminergic Agonists

Intravenous administration of saline failed to evoke compulsive gnawing in naive rabbits. Apomorphine, 4.0 mg/kg, IV, induced this stereotyped behavior in 8 of 28 rabbits (29%). Administration of this dose of apomorphine to different animals pretreated with pCPA, 3 × 300 mg/kg,

IV, or cyproheptadine, 2.0 mg/kg, IV, produced gnawing in 4 of 12 (33%) and 7 of 28 (25%) rabbits, respectively. These incidences were not significantly different from the apomorphine controls.

d-Amphetamine, 5.0 mg/kg, IV, evoked compulsive gnawing in 6 of 26 rabbits (23%). However, in contrast to the apomorphine experiments, d-amphetamine produced stereotyped behavior in 6 of 12 animals (50%) following pCPA pretreatment and also induced gnawing in 5 of 6 cyproheptadine-pretreated rabbits (83%). Both these incidences were significantly different from the d-amphetamine control group ($p<0.05$ and $p<0.01$, respectively).

Influence of Various Drug Pretreatments on Rabbit Brainstem Serotonin Content

Seventeen naive rabbits were sacrificed and their brainstems were removed and assayed for serotonin; their mean brainstem serotonin content was 0.58 ± 0.02 μg/g ± SEM. Thirty-five other brainstem sections were removed from drug-pretreated animals at times following drug pretreatment when they would normally have received either apomorphine or d-amphetamine. Twenty-one brainstem samples from rabbits pretreated with pCPA, 3 × 300 mg/kg, IP, showed a marked reduction in the serotonin content down to 0.15 ± 0.01 μg/g; this was significantly different from the control group ($p<0.01$). In 7 pCPA-pretreated rabbits also receiving Carbidopa, 25 mg/kg, IP, and 5-HTP, 10 mg/kg, IV, brainstem serotonin content was restored to 0.61 ± 0.03 μg/g. Pretreatment with cyproheptadine, 2.0 mg/kg, IV, failed to significantly change the brainstem serotonin content of 7 rabbits; serotonin levels remained at 0.58 ± 0.03 μg/g.

DISCUSSION

Our experimental findings have uncovered a difference in the influence of serotonergic antagonists upon the neuropharmacological properties of apomorphine and d-amphetamine. Pretreatment with pCPA reduces brainstem serotonin content and simultaneously abolishes the hyperthermic response to apomorphine. However, pretreatment reduces the magnitude of the d-amphetamine-induced hyperthermia by only 50%. Both temperature responses were restored either in part of in toto following regeneration of central serotonin levels prior to a second challenging indection of either apomporphine or d-amphetamine. These findings suggest that serotonin might be involved in both the apomorphine- and d-amphetamine-induced hyperthermic responses. A major objection to this interpretation, however, is that pCPA in high doses reduces brain catecholamine synthesis as well as serotonin synthesis [15,17]. Therefore, we attempted to reproduce these findings with serotonergic receptor blockers.

The serotonergic antagonists cyproheptadine and cinanserin effectively reduced the temperature response to apomorphine but not to d-amphetamine. Higher doses of the pretreatment drugs were not used since they produced a marked sedative and prolonged hypothermic effect in the animals. Preliminary studies with the neurotoxic agent 5,6-dihydroxytryptamine also showed antagonism of the apomorphine but not the d-amphetamine response. Why the apomorphine-induced hyperthermia should be more sensitive to serotonergic antagonists is not known. However, it may be possible that apomorphine and d-amphetamine-released dopamine activate two separate dopaminergic substrates in the rabbit central nervous system and that serotonin is more intimately involved in the apomorphine-activated pathway. This view is supported by the recent report of Snow and Horita [22] which indicates that the hyperthermic action of apomorphine occurs only under stanchion restraint conditions, whereas d-amphetamine produces its temperature effect in the presence or absence of stanchion restraint.

The present study also suggests that serotonin is involved in the locomotor stimulation evoked by d-amphetamine but not the motor response to apomorphine. Serotonergic antagonists failed to block apomorphine-induced hyperkinesia, although the hypoactive component of the response was abolished. Similar doses of these antagonists all significantly reduced d-amphetamine-induced locomotor hyperactivity; furthermore, locomotor stimulation reappeared in pCPA-pretreated rabbits after restoration of central serotonin levels. These data are different than previous reports of experiments conducted in rodents where serotonin has been reported to exert an inhibitory influence upon apomorphine- or d-amphetamine-induced locomotor stimulation [2, 3, 9, 10, 16].

Our findings also show that serotonin is involved in inhibition of d-amphetamine-induced compulsive gnawing but may have no role in the stereotypic response to apomorphine. This is in marked contrast to data obtained in rodents in which serotonergic antagonists or midbrain raphe lesions either reduce or have no effect upon dopaminergic agonist-induced stereotyped behavior [6, 14, 20, 23].

In summary, the present study has demonstrated that the neuropharmacological actions of apomorphine and d-amphetamine in the rabbit (a) exhibit differences in their interactions with serotonin synthesis inhibitors and receptor blockers; (b) are not mechanistically similar to the actions seen in rodents; and (c) cannot be considered identical although they both act via dopaminergic receptor mechanisms.

REFERENCES

1. Bogdanski, D. F., A. Pletscher, B. B. Brodie and S. Udenfriend. Identification and assay of serotonin in brain. *J. Pharmac. exp. Ther.* **117:** 82–88, 1956.
2. Breese, G. R., B. R. Cooper and A. S. Hollister. Relationship of biogenic amines to behavior. *J. Psychiat. Res.* **11:** 125–133, 1974.
3. Breese, G. R., B. R. Cooper and R. A. Mueller. Evidence for involvement of 5-hydroxytryptamine in the actions of amphetamine. *Br. J. Pharmac.* **52:** 307–314, 1974.
4. Bunney, B. S., G. K. Aghajanian and R. H. Roth. Comparison of effects of L-DOPA, amphetamine and apomorphine on firing rate of dopaminergic neurones. *Nature* **245:** 123–135, 1973.
5. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacology* **10:** 316–323, 1967.
6. Ernst, A. M. Relationship of the central effect of dopamine on gnawing compulsion syndrome in rats and the release of serotonin. *Arch. int. Pharmacodyn.* **199:** 219–225, 1972.

7. Ernst, A. M. and P. G. Smelik. Site of action of dopamine and apomorphine on compulsive gnawing behavior in rats. *Experientia* **22:** 837–838, 1966.
8. Goldstein, A. *Biostatistics: An Introductory Text.* New York: Macmillan Company, 1964, pp. 107–110.
9. Grabowska, M., L. Antikiewicz, J. Maj and J. Michaluk. Apomorphine and central serotonin neurons. *Pol. J. Pharmac. Pharm.* **25:** 29–39, 1973.
10. Grabowska, M. and J. Michaluk. On the role of serotonin in apomorphine-induced locomotor stimulation in rats. *Pharmac. Biochem. Behav.* **2:** 263–266, 1974.
11. Hill, H. F. *The Production of Hyperthermia in the Rabbit by an Action of D-Amphetamine on Central Dopaminergic Neurons.* Ph.D. Dissertation, University of Washington, Seattle, 1971.
12. Hill, H. F. and A. Horita. Inhibition of D-amphetamine hyperthermia by blockade of dopamine receptors in rabbits. *J. Pharm. Pharmac.* **23:** 715–717, 1971.
13. Hill, H. F. and A. Horita. A pimozide-sensitive effect of apomorphine on body temperature of the rabbit. *J. Pharm. Pharmac.* **24:** 490–491, 1972.
14. McKenzie, G. M., K. Viik and C. E. Boyer. Selective blockade of apomorphine-induced aggression and gnawing following fenfluramine or raphe lesions in the rat. *Fedn Proc.* **32:** 248, 1973.
15. Miller, F. P., R. H. Cox, W. R. Snodgrass and R. P. Maickel. Comparative effects of p-chlorophenylalanine, p-chloroamphetamine and p-chloro-N-methyl-amphetamine on rat brain norepinephrine, serotonin and 5-hydroxy-indole-3-acetic acid. *Biochem. Pharmac.* **19:** 435–442, 1970.
16. Neill, D. B., L. D. Grant and S. P. Grossman. Selective potentiation of locomotor effects of amphetamine by midbrain raphe lesions. *Physiol. Behav.* **9:** 655–657, 1972.
17. Peters, D. A. V., M. Filczewski and I. M. Mazurkiewicz-Kwilecki. Effect of parachlorophenylalanine on catecholamine synthesis in rat brain, heart and adrenals. *Biochem. Pharmac.* **21:** 2282–2284, 1972.
18. Quock, R. M., M. A. Carino and A. Horita. Thermotropic activity of apomorphine in the rabbit: a central site of action. *Life Sci.* **16:** 525–532, 1975.
19. Quock, R. M. and A. Horita. Apomorphine: modification of its hyperthermic effect in rabbits by p-chlorophenylalanine. *Science* **183:** 539–540, 1974.
20. Rotrosen, J., B. M. Angrist, M. B. Wallach and S. Gershon. Absence of serotonergic influence on apomorphine-induced stereotypy. *Eur. J. Pharmac.* **20:** 133–135, 1972.
21. Shellenberger, M. K. and J. T. Elder. Changes in rabbit core temperature accompanying alterations in brainstem monoamine concentrations. *J. Pharmac. exp. Ther.* **158:** 219–226, 1967.
22. Snow, A. and A. Horita. The stress dependent nature of apomorphine hyperthermia in the rabbit. *Proc. West. Pharmac. Soc.* **18:** 43–45, 1975.
23. Tice, P. and C. E. Spooner. Antagonism of d-amphetamine by methysergide. *Pharmacologist* **12:** 226, 1970.