increased to 893 ± 104 pmol mg^{-1} tissue protein, whereas the mean vasopressin release reached 15·1 ± 1·8%. These values were significantly higher ($P < 0.05$) than those observed in control glands, but were not different from those obtained with glands incubated in the presence of angiotensin II alone.

Our previous findings that inhibition of the converting enzyme by SQ 20881 could prevent the increase in cyclic AMP content and vasopressin release induced by angiotensin I indicated that the decapeptide was probably transformed into an octapeptide (angiotensin II) before producing its neurohypophyseal effects (Sirois & Gagnon, 1975). It was then assumed that this inhibitory effect was not due to a direct antagonism of the neurohypophyseal receptor site for angiotensin by SQ 20881, as evidenced by Ng & Vane (1970) and Engel & others (1972) who demonstrated that the nonapeptide did not affect the response to angiotensin mediated by peripheral receptor sites. The present results demonstrated clearly that SQ 20881 does not interfere with the binding of angiotensin to its neurohypophyseal receptors. They also provided further evidence as to the role of the converting enzyme in the neurohypophyseal actions of angiotensin I.

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REFERENCES

COUSINEAU, D., GAGNON, D. J. & SIROIS, P. (1973). *Br. J. Pharmac.*, **47**, 315–324.
ENGEL, S. L., SCHAEFFER, I. R., GOLD, B. I. & RUBIN, B. (1972). *Proc. Soc. exp. Biol. Med.*, **140**, 240–244.
GAGNON, D. J., COUSINEAU, D. & BOUCHER, P. J. (1973). *Life Sci.*, **12**, 487–497.
GAGNON, D. J. & HEISLER, S. (1974). *Biochim. biophys. Acta*, **338**, 394–397.
GAGNON, D. J., SIROIS, P. & BOUCHER, P. J. (1975a). *Clin. exp. Pharmac. Physiol.*, **2**, 305–313.
GAGNON, D. J., SIROIS, P. & PARK, W. K. (1975b). *Ibid.*, **2**, 315–322.
GILMORE, N. J. & VANE, J. R. (1970). *Br. J. Pharmac.*, **38**, 633–652.
LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDAL, R. J. (1951). *J. biol. Chem.*, **193**, 265–275.
NG, K. K. F. & VANE, J. R. (1970). *Nature, Lond.*, **225**, 1142–1144.
SIROIS, P. & GAGNON, D. J. (1975). *J. Neurochem.*, **25**, 727–729.
TSANG, C. P. W., LEHOTAY, D. C. & MURPHY, B. E. P. (1972). *J. clin. Endocr. Metab.*, **35**, 809–817.
YANG, H. Y. T. & NEFF, N. H. (1972). *J. Neurochem.*, **19**, 2443–2450.
YANG, H. Y. T. & NEFF, N. H. (1973). *Ibid.*, **21**, 1035–1036.

Comparison of the dopaminergic effects of *N*-substituted aporphines

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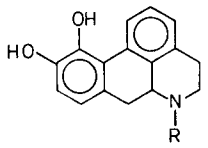
Because apomorphine has short duration of action, it causes depression and is ineffective on oral administration with development of azetomia on increasing its dose, its therapeutic usefulness (Cotzias, Lawrence & others, 1972) in alleviating Parkinsonian symptoms is limited. The possibility of the development of long-acting analogues with minimal emetic effects and longer duration of dopaminergic effects than the parent drug, still exists. Since earlier studies of many investigators (Koch, Cannon & Burkman 1968; Pinder, Buxton & Green 1971; Lal, Sourkes & others, 1972; Neumeyer, McCarthy & others, 1973a; Neumeyer, Neustadt & others, 1973b; Neumeyer, Granchelli & others, 1974; Saari & King, 1974) showed that changes in ring substitution reduced or abolished the dopaminergic effects of the parent compound, we selected 4 apomorphine derivatives differing from apomorphine only in their *N*-substituent. Activation of reserpinized mice was used as the criterion for evaluation of their dopaminergic effects and apomorphine was used as the reference standard. Since one of the main drawbacks of apomorphine is its emetic effect, its interaction with two antiemetics also has been investigated.

Male Swiss mice (23–28 g, Horton Labs) maintained at 23 ± 1° were pretreated with reserpine (5 mg kg^{-1}) 4 h before the experiment. Various doses of the apomorphines were administered and immediately after injection, the amounts were placed individually in plastic chambers (12·5 cm^3) on an activity meter (Model 2S, Columbus Instrument Company, Ohio) and their motor activity was recorded. The aporphines tested were the hydrochlorides of (—)-apomorphine, (±)-norapomorphine, (±)-ethylnorapomorphine, (±)-propylnorapomorphine and (±)-methylcyclopropylnor-

† Correspondence.



	R
Apomorphine	$-CH_3$
Norapomorphine	$-H$
N-Ethylnorapomorphine	$-CH_2CH_3$
N-n-Propylnorapomorphine	$-CH_2CH_2CH_3$
N-Methylcyclopropylnorapomorphine	$-CH_2-\triangleleft$

apomorphine. For one experiment (—)-propylnorapomorphine was used. The solutions of the aporphines were freshly prepared in distilled water containing 0·01% ascorbic acid. The antiemetics used were metoclopramide (dissolved in distilled water) and sulpiride (dissolved in 0·01 N HCl). All the drugs were administered intraperitoneally in a volume of 0·01 ml g^{-1} body weight. The doses represent the salt form.

Mice treated with reserpine were highly sedated. They showed the characteristic hunch-back posture, rigidity and ptosis.

In doses effective in antagonizing reserpine sedation, the behavioural effects produced by the apomorphine analogues were qualitatively similar to those of apomorphine. In a dose of 1 mg kg^{-1} apomorphine and the ethyl and n-propyl derivatives antagonized reserpine depression within 3 min. The animals moved about with normal gait which became jerky at times. Most of the animals showed stereotyped behaviour consisting of sniffing and biting the sides of the compartments. Unexpectedly, in higher doses, all these compounds were capable of producing moderate hind limb rigidity. In the case of apomorphines 40% of the animals showed this effect in doses above 100 mg kg^{-1}. However, the n-ethyl and n-propyl derivatives produced rigidity in 40–50% of the animals even in a dose of 3 mg kg^{-1}. Higher doses did not seem to further enhance this response. Norapomorphine antagonized reserpine's effect only at 30 mg kg^{-1} when the effect was seen within 3 min. The behavioural responses were similar to those described above except that hind limb rigidity was absent even at a dose of 100 mg kg^{-1}. The methylcyclopropyl analogue, although reversing the reserpine effect, failed to produce locomotor stimulation. Hind limb rigidity was evident in 80% of the animals treated with a dose of 10 mg kg^{-1}. At a dose of 30 mg kg^{-1} intermittent clonic convulsions and persistent extension of both the pairs of limbs in a rigid manner were seen. In spite of these effects, at doses above 3 mg kg^{-1}, this compound produced stereotyped head movements and biting.

Reserpine-induced ptosis was not antagonized by any of these compounds.

Since these compounds exerted their stimulant effects within 3 min and the maximal effect was over in 60 min, the initial 60 min activity was used to compare the potencies of the various derivatives (Fig. 1). Apomorphine and the ethyl and n-propyl derivatives showed marked antireserpine effects even at a dose of 1 mg kg^{-1}. At this dose, apomorphine and the n-propyl derivatives were equipotent, whereas the ethyl derivative was about 50% more active. All three compounds exerted maximal effects at 10 mg kg^{-1} when they were about equipotent. Further increase in the dose reduced the motor responses mainly due to the persistent stereotyped behaviour leading to a decrease in the locomotor effects. Norapomorphine was ineffective up to 10 mg kg^{-1}, but showed prominent motor stimulation at 30 and 100 mg kg^{-1}. The response produced by this compound in a dose of 100 mg kg^{-1} compared favourably with the effects of the other 3 compounds at 100 mg kg^{-1}. The methylcyclopropyl derivative showed only minimal effectiveness in reversing the reserpine effect and produced toxic effects when the dose exceeded 10 mg kg^{-1}.

In comparing the locomotor stimulation produced by apomorphine and the ethyl and n-propyl derivatives at a dose of 10 mg kg^{-1}, and, taking into consideration the fact that the (—)-isomer is the active form (Saari, King & Lotti, 1973; Schoenfeld, Neumeyer & others, 1975) it is concluded that the n-ethyl and n-propyl derivatives are twice as potent as apomorphine in reversing reserpine depression. Norapomorphine is 5–15 times less active than apomorphine (depending on whether comparison was made with 3 mg kg^{-1} or 10 mg kg^{-1} apomorphine), whereas the methylcyclopropyl substitution markedly reduced the antireserpine effect and also enhanced the acute toxicity.

Recent reports indicating (—)-propylnorapomorphine

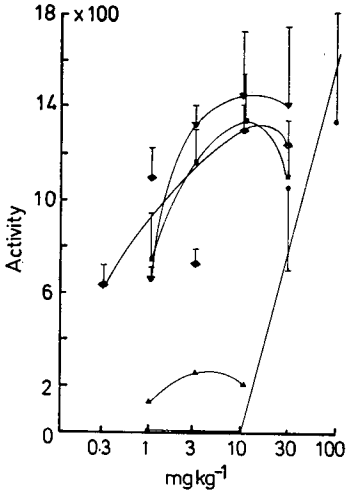


FIG. 1. Different doses of the various aporphine derivatives were administered intraperitoneally to mice pretreated with reserpine (5 mg kg^{-1}, i.p. 4 h previously) and their locomotor activity was recorded for 60 min. ▼ Apormorphine, ● norapomorphine, ◆ ethylapomorphine, ■ n-propylapomorphine, ▲ methylcyclopropylapomorphine. y axis—60 min activity meter readings ± s.e. x axis—Log dose (mg kg^{-1}).

to be many times more effective than apomorphine in eliciting stereotyped behaviour in rats (Costall, Naylor & Neumeyer, 1975; Schoenfeld & others, 1975) necessitated the comparison of the potency of these two compounds in the present model. However, we did not find any superior effects of the propyl derivative. The 60 min motor activity reading obtained after a 1 mg kg^{-1} dose of (—)-propylapomorphine was 475 ± 119 which was less than that obtained after a similar dose of apomorphine (660 ± 47). At this dose, the maximal effects (locomotion and stereotypy) of both these compounds were over at 60 min. Both drugs produced stereotyped sniffing and biting of apparently similar intensity. However, 70% of the animals treated with the propyl derivative showed hind limb rigidity for the initial 30 min. Such an effect was not observed in the apomorphine-treated group. The 60 min activity produced by 0·1 mg kg^{-1} of the (—)-propyl derivative was only 25% of the response of 1 mg kg^{-1} of apomorphine.

The antireserpine effect of apomorphine was not prolonged beyond 60 min even when the dose was increased to 30 mg kg^{-1}. On the other hand, increasing the dose led to more persistent effects in the case of norapomorphine, and the ethyl and propyl derivatives. In a dose of 30 mg kg^{-1}, these compounds acted for 120, 150 and 120 min respectively.

Norapomorphine 100 mg kg^{-1} (equivalent to 50 mg kg^{-1} of the (—)-form) caused intense motor stimulation lasting for 150 min and, even after 240 min, residual effects were still apparent. Thus, though this compound is 5–15 times less active than apomorphine, its duration was 4 times longer than that of an equipotent dose of apomorphine (10 mg kg^{-1}).

The maximal effects of methylcyclopropyl derivative were over by 30 min. Though the animals looked alert and became active when touched, the absence of any running behaviour precluded the determination of its duration of action.

In reserpinized mice, pretreatment with metoclopramide (3 mg kg^{-1}) 15 min before apomorphine completely abolished apomorphine effects. Sulpiride (1 mg kg^{-1}) 15 min previously), did not modify either the stereotypy or locomotor stimulation caused by a similar dose of apomorphine. The 30 min locomotor effect of apomorphine was 624 ± 44 and in the sulpiride treated animals it was 584 ± 19.

When the dopaminergic effects of the *N*-substituted aporphines studied were evaluated on the basis of their antireserpine effects, it was found tha n-ethyl and n-propyl substituents increase the potency two fold and produced more persistent effects than apomorphine. Absence of an alkyl group at the N led to reduced potency, but prolonged its antireserpine effects. The methylcyclopropyl substitution markedly reduced the antireserpine effect. Though this compound reduced stereotyped searching behaviour, unlike the other aporphines it failed to produce motor stimulation.

Of the aporphine derivatives, that receiving most attention in recent years has been the n-propyl one, investigators believing the derivative to be more effective than apomorphine and for which activity equivalent to as much as 40 times greater than apomorphine has been reported (Koch & others, 1968; Atkinson, Bullock & Granchelli, 1975; Costall & others, 1975; Mendez, Cotzias & others, 1975; Schoenfeld & others, 1975) depending on the parameters used.

Our animals had been pretreated with reserpine which does not interfere with the dopaminergic effects of apomorphine. Some reports show the apomorphine effect to be enhanced in reserpinized animals (own observation; Patni & Dandiya, 1974; Butterworth, Poignant & Barbeau, 1975) the hind limb rigidity caused by the n-propyl derivative could have interfered with the locomotor response. This possibility was eliminated when it was found that at 0·1 mg kg^{-1} it failed to evoke rigidity in mice and was only one quarter as active as apomorphine.

Mendez & others (1975) observed that the rotatory behaviour in nigra-lesioned rats produced by n-propylapomorphine could be considerably prolonged by atropine. They also cite other evidence for the cholinergic effects of this compound. A stimulation of the cholinergic system would be more evident in a model in which the opposing dopaminergic system is functioning at a subnormal state. The hind limb rigidity shown by some of the apomorphine analogues in reserpinized mice could be explained on this basis. Norapomorphine, the longer acting compound, is devoid of such an effect. From this it seems that *N*-substitution confers this property to the molecule.

From the therapeutic point of view, the usefulness of an apomorphine analogue will be diminished, if the increase in the dopaminergic activity is accompanied by a proportionate increase in its emetic potency.

One way to deal with this problem would be to administer antiemetics which do not interfere with the dopaminergic effect of the compound. Our interaction studies with antiemetics showed that, while metaclopramide completely blocked the dopaminergic effect of apomorphine, sulpiride did not modify this response. Metoclopramide is a powerful antiemetic (Laville, 1964, Malmejac, Laville & Margarit, 1964) and has the profile of a neuroleptic in that, it produces catalepsy in rats (Costall & Naylor, 1973), antagonizes apomorphine-induced compulsive gnawing (Hackman, Pentikäinen & others, 1973) and increases brain homovanillic acid concentrations of rats (Ahtee & Buncombe, 1974; Peringer, Jenner & Marsden, 1975). Hence it was not surprising to find that in the present studies this drug blocked the effect of apomorphine making it unsuitable as an antiemetic drug in conjunction with apomorphine.

Sulpiride, a newly introduced neuroleptic agent structually related to metoclopramide and possessing powerful antiemetic effects, does not produce extrapyramidal disturbances in man and has not been found

to block the dopaminergic effects of either amphetamine or apomorphine (Laville & Margarit, 1968a, b; Kato, Sato & Shimomura, 1974). We found sulpiride failed to block apomorphine effects in reserpinized mice. Therefore it might prove to be beneficial in blocking apomorphine-induced emesis without interfering with its beneficial effects in Parkinson's disease. In this regard the report of Gessa, Gessa & others (1975) that both metoclopramide and sulpiride selectively block the emetic effect of apormorphine in man without interfering with its antiparkinsonism effect is encouraging.

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REFERENCES

AHTEE, L. & BUNCOMBE, G. (1974). *Acta pharmac. tox.*, **35**, 429–432.

ATKINSON, E. R., BULLOCK, F. J. & GRANCHELLI, F. E. (1975). *J. medl Chem.*, **18**, 1000–1003.

BUTTERWORTH, R. E., POIGNANT, J. C. & BARBEAU, A. (1975). In: *Adv. Neurol.*, **9** Pp. 307–326. (D. Calne, T. N. Chase and A. Barbeau, eds.). New York: Raven Press.

COSTALL, B. & NAYLOR, R. J. (1973). *Arzneimitt.-Forsch.*, **23**, 674–683.

COSTALL, B., NAYLOR, R. J. & NEUMEYER, J. L. (1975). *Eur. J. Pharmac.*, **31**, 1–16.

COTZIAS, G. C., LAWRENCE, W. H., PAPAVASILIOU, P. S., DÜBY, S. W., GRINOS, J. Z. & MENA, I. (1972). *Trans. Am. Neurol. Assoc.*, **97**, 156–159.

GESSA, G. L., GESSA, R., STEFANINI, E., PORCEDDU, M. L. & CORSINI, G. U. (1975). *Abstr. 6th Int. Congr. Pharmac., Helsinki, July* 20–25. Abstr. No. 1148, p. 486.

HACKMAN, R., PENTIKÄINEN, P., NEUVONEN, P. J. & VAPAATALO, H. (1973). *Experientia*, **29**, 1524–1525.

KATO, R., SATO, Y. & SHIMOMURA, K. (1974). *Jap. J. Pharmac.*, **5**, *Suppl.* 2, 48–49.

KOCH, M. V., CANNON, J. G. & BURKMAN, A. M. (1968). *J. medl Chem.*, **11**, 977–981.

LAL, S., SOURKES, T. L., MISSALA, K. & BELENDINK, G. (1972). *Eur. J. Pharmac.*, **20**, 71–79.

LAVILLE, C. (1964). *Path. Biol. (Paris)*, **12**, 577–578.

LAVILLE, C. & MARGARIT, J. (1968a). *Ibid.*, **16**, 13, 14.

LAVILLE, C. & MARGARIT, J. (1968b). *Compt. rend. Soc. Biol.*, **162**, 869–874.

MALMEJAC, J., LAVILLE, C. & MARGARIT, J. (1964). *Ibid.*, **158**, 964–965.

MENDEZ, J. S., COTZIAS, G. C., FINN, B. W. & DAHL, K. (1975). *Life Sci.*, **16**, 1737–1742.

NEUMEYER, J. L., MCCARTHY, M., BATTISTA, S. P., ROSENBERG, F. J. & TEIGER, D. G. (1973a). *J. medl Chem.*, **16**, 1228–1233.

NEUMEYER, J. L., GRANCHELLI, F. E., FUXE, K., UNGERSTEDT, U. & CORRODI, H. (1974). *Ibid.*, **17**, 1090–1095.

NEUMEYER, J. L., NEUSTADT, B. R., OH, K. H., WEINHARDT, K. K., BOYCE, C. B., ROSENBERG, F. J. & TEIGER, D. G. (1973b). *Ibid.*, **16**, 1223–1228.

PATNI, S. K. & DANDIYA, P. C. (1974). *Life Sci.*, **14**, 737–745.

PERINGER, E., JENNER, P. & MARSDEN, C. D. (1975). *J. Pharm. Pharmac.*, **27**, 442–444.

PINDER, R. M., BUXTON, D. A. & GREEN, D. M. (1971). *Ibid.*, **23**, 995–996.

SAARI, W. S., KING, S. W. & LOTTI, V. G. (1973). *J. medl Chem.*, **16**, 171–172.

SAARI, W. S. & KING, S. W. (1974). *Ibid.*, **17**, 1086–1090.

SCHOENFELD, R. I., NEUMEYER, J. L., DAFELDECKER, W. & ROFFLER-TARLOV, S. (1975). *Eur. J. Pharmac.*, **30**, 63–68.

TAGLIAMONTE, A., DEMONTIS, G., OLIANAS, M., VARGIN, L., CORSINI, G. U. & GESSA, G. L. (1975). *J. Neurochem.*, **24**, 707–710.