throline metal chelates may be the $Ca^{2+}$-dependent stimulus-secretion coupling mechanism. Moreover, the negative chronotropic action of TMP (Figure 1) may reflect its ability to immobilize $Ca^{2+}$ ions by chelation. The actions on the atrial preparation of a variety of fully co-ordinated chelates differing only in the transition metal ion or in the ligand will be reported fully elsewhere.

*Zusammenfassung.* Nachweis, dass vollkoordinierte zweiwertige Kupfer- und Nickelchelate des 3,4,7,8-Tetramethyl-1,10-phenanthrolins Muskelkraft, wie auch Kontraktionsgeschwindigkeit des isolierten Meerschweinchen Atriums, sowie $H^3$-Noradrenalin- und Metabolitenausfluss vermehren. Die durch Chelate veranlasste Senkung des Noradrenalins konnte durch Fluoreszenz-Histochemie bestätigt werden.

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[8] This work was commenced in the Department of Pharmacology, University of Melbourne and was presented in part by H. G. in November 1972 in partial fulfilment for the degree of B. Sc. (Hons) in the University of Melbourne.

## Inhibition of the Apomorphine Gnawing Compulsion by Amantadine

A hundred years ago HARNACK[1] reported that apomorphine in rats induces a compulsive gnawing behaviour, which has been suggested to be due to direct action on the dopaminergic receptors[2–4]. Amantadine is beneficial in the treatment of parkinsonism and has central stimulatory properties, but the underlying mechanisms are not fully resolved[5–6]. In order to study the mode of central action of amantadine, the apomorphine gnawing test was used. The effects of amantadine were compared to those of some drugs known to act on dopaminergic receptors.

Male Spraque-Dawley rats (230–280 g) were given apomorphine 30 min after treatment with various doses of chlorpromazine, metoclopramide or amantadine, or simultaneously with L-Dopa. After a further interval of 30 min, the intensity of the gnawing compulsion was noted using an arbitrary scale from 0 to 3 (0, no gnawing; 1, slight; 2, moderate and 3, intense gnawing).

Apomorphine caused a dose-dependent gnawing compulsion (Figure 1). L-Dopa (50 and 100 mg/kg i.p.) did not induce gnawing during the observation period of 2 h, but potentiated the effect of apomorphine (Table I). Chlorpromazine, metoclopramide and amantadine inhibited dose-dependently the gnawing induced by apomorphine (Figure 2). The inhibition caused by metoclopramide in a dose of 0.5 mg/kg i.p. seemed to be non-competitive (Figure 1).

Apomorphine stimulates dopaminergic receptors even more effectively than dopamine itself[3] and this probably evokes the gnawing compulsion. Accordingly it is under-

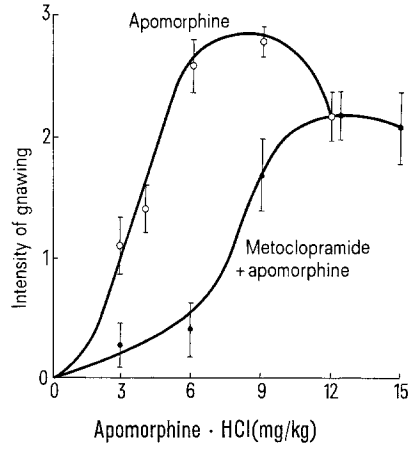


Fig. 1. Inhibition of apomorphine-gnawing by metoclopramide. Metoclopramide (0.5 mg/kg i.p.) or saline were injected 30 min before the administration of various i.p. doses of apomorphine. The gnawing compulsion was noted after a further 30 min using an intensity scale from 0 to 3. The means ± S.E. of 10 rats are given.

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[2] N.-E. ANDEN, A. RUBENSON, K. FUXE and T. HÖKFELT, J. Pharm. Pharmac. *19*, 627 (1967).
[3] A. M. ERNST, Psychopharmacologia *10*, 316 (1967).
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Table I. Effect of L-Dopa on apomorphine gnawing in rats

| Drugs and dosage | Gnawing (scale 0–3) |
|---|---|
| Saline + saline | 0 ± 0 |
| Saline + L-Dopa (50 mg/kg) | 0 ± 0 |
| Saline + L-Dopa (100 mg/kg) | 0 ± 0 |
| Apomorphine (4 mg/kg) + saline | 1.4 ± 0.3 |
| Apomorphine (4 mg/kg) + L-Dopa (50 mg/kg) | 1.8 ± 0.4 |
| Apomorphine (4 mg/kg) + L-Dopa (100 mg/kg) | 2.7 ± 0.2[a] |

L-Dopa and apomorphine or saline were given simultaneously i.p. and the gnawing compulsion was noted after a further 30 min. Means ± S.E. are given ($n = 5$–8). [a] $p < 0.05$ when compared to the apomorphine-saline group.

Table II. Effect of amantadine on apomorphine gnawing in rats

| Drug and dosage | Gnawing (scale 0–3) |
|---|---|
| Saline + apomorphine | 1.4 ± 0.2 |
| Amantadine (1.0 mg/kg) + apomorphine | 1.6 ± 0.5 |
| Amantadine (2.5 mg/kg) + apomorphine | 1.4 ± 0.4 |
| Amantadine (5.0 mg/kg) + apomorphine | 0.9 ± 0.2 |
| Amantadine (10.0 mg/kg) + apomorphine | 0.8 ± 0.3 |
| Amantadine (25.0 mg/kg) + apomorphine | 0.6 ± 0.2[a] |
| Amantadine (50.0 mg/kg) + apomorphine | 0 ± 0[a] |

Saline or amantadine was given i.p. 30 min before the i.p. administration of apomorphine (4 mg/kg). Gnawing compulsion was noted after a further 30 min. Means ± S.E. are given ($n = 6$–13). [a] $p < 0.05$ when compared to the saline-apomorphine group.

standable that L-Dopa enhanced the effects of apomorphine. Amantadine, on the contrary, inhibited the gnawing compulsion induced by a dose of 10 mg/kg of apomorphine. A similar dose-dependent inhibition by amantadine was observed when a dose of apomorphine (4 mg/kg) inducing half the maximal effect was used (Table II). Amantadine behaved in this respect as the dopaminergic receptor blocking agents, metoclopramide and chlorpromazine.

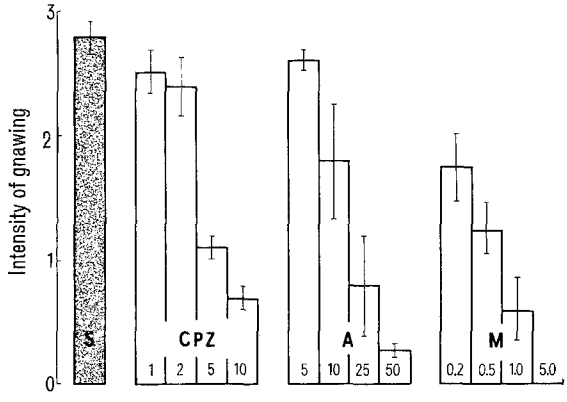


Fig. 2. Effect of chlorpromazine (CPZ), amantadine (A), metoclopramide (M) or saline (S) on the gnawing compulsion induced by apomorphine. CPZ, A, M or S were given in the doses indicated (mg/kg i.p.) 30 min before the injection of apomorphine (10 mg/kg) and the gnawing compulsion was noted after a further 30 min using an arbitrary scale from 0 to 3. The means ± S.E. of 5–10 rats are given.

The inhibition of apomorphine gnawing by amantadine cannot be explained only by the release of dopamine, which has been suggested as a mechanism for the action of amantadine[7]. We propose that amantadine, in addition to its amine releasing properties, has the ability partially to occupy the dopaminergic receptors without causing a marked agonistic action of its own, and thus competes with apomorphine at the receptor sites.

*Zusammenfassung.* Amantadin bewirkte bei Ratten eine dosisabhängige Hemmung der sogenannten Apomorphin-«Gnawing Compulsion» und verhielt sich somit ähnlich wie ein Dopaminrezeptorblocker.

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[7] R. P. Grelak, R. Clark, J. M. Stump and V. G. Vernier, Science *169*, 203 (1970).

## Catecholamine Depleting Effect of Black Widow Spider Venom on Fibres Innervating Different Guinea-Pig Tissues

The venom of the black widow spider *Latrodectus mactans tredecimguttatus* (Rossi) has been shown to cause acetylcholine (ACh) depletion of rat superior cervical ganglia[1], of brain cortex slices[2] and of *Torpedo* electric tissue slices[3], when added in vitro to the tissue incubation medium. These findings are in good agreement with electrophysiological experiments where the venom was assayed in vitro on cholinergic systems such as frog[4] and cat[5] muscle end-plates and rat superior cervical ganglia[1,6], indicating a presynaptic site of action of the venom, affecting the mechanism of transmitter release. *Latrodectus* venom has been demonstrated to affect also other types of nerve endings besides the cholinergic ones, namely, lobster excitatory and inhibitory neuromuscular junctions[7], locust excitatory neuromuscular junctions[8] and rat iris adrenergic nerve-terminals[9]. The last result was obtained by subjecting irises incubated in vitro to fluorescence histochemistry for the detection of catecholamines; addition of the venom was followed by the disappearance of the specific yellow-green fluorescence of the adrenergic nerve fibres. This catecholamine-depleting effect was not secondary to ACh release, as demonstrated by the normal appearance of controls where irises were incubated in the presence of ACh and eserine or of carbachol.

Extending the observations on rat iris, we have now assayed the venom on thin innervated tissues of guinea-pig, equally suited for stretch preparations. In addition to irises, fragments of mesentery, spleen capsule, inferior vena cava, gut longitudinal muscular layer (including

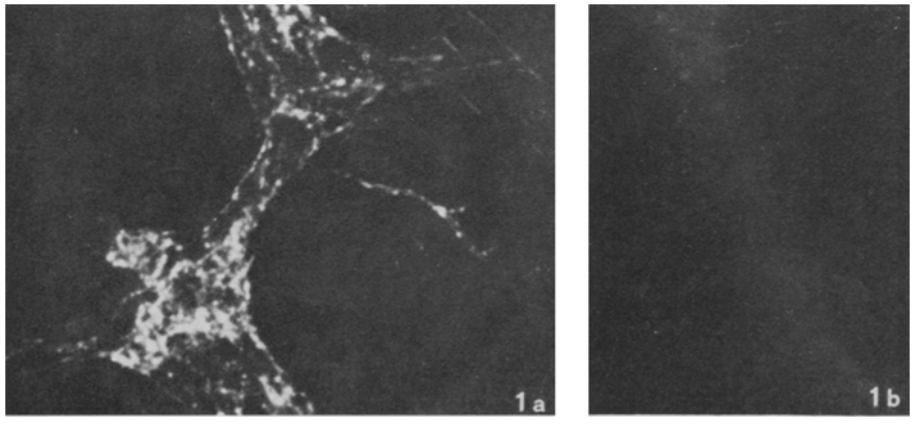


Fig. 1. Longitudina muscular layer of guinea-pig gut incubated in the absence (a) and in the presence (b) of the venom (0.5 pairs of glands/ml of medium). The yellow-green fluorescence of the adrenergic nerve fibres forming the Auerbach plexus disappears as a consequence of venom addition.