Action of Fully Co-Ordinated 1,10-Phenanthroline Transition Metal Chelates on the Guinea-Pig Isolated Atrium

Fully co-ordinated divalent 1,10-phenanthroline transition metal chelates have cholinolytic action at both nicotinic and muscarinic receptors. They also liberate acetylcholine from the guinea-pig isolated ileum and block α-adrenergic transmission in the rat isolated vas deferens preparation[1-4]. The present investigation shows that such chelates also liberate noradrenaline from the β-adrenergic guinea-pig isolated atrium.

Materials and methods. The actions of *bis*(3,4,7,8-tetramethyl-1,10-phenanthroline)copper(II) diacetate (CuTMP) and *tris*(3,4,7,8-tetramethyl-1,10-phenanthroline)nickel(II) sulphate (NiTMP) were compared with those of their constituent divalent metal ions Cu(II) and Ni(II) (as acetates), and ligand 3,4,7,8-tetramethyl-1,10-phenanthroline (TMP) (as the hydrochloride). The atrial preparation was suspended in Krebs-Henseleit solution at 30 °C and gassed with 5% carbogen. The spontaneous force and rate of contraction were recorded by means of a transducer and an electronic pen recorder. Following a 20 min preincubation of the atrial preparation with 7-[^{3}H]-noradrenaline (5 μCi/ml; 1.25×10^{-6} *M*), the spontaneous or drug-induced efflux of tritiated materials was determined by sampling the bath contents at 2 min intervals and estimating the amount of tritium present by liquid scintillation spectrometry. Chelate-induced release of endogenous noradrenaline was also examined by fluorescence histochemistry[5].

Results and discussion. CuTMP and Cu(II) ion (1×10^{-4} *M* for 30 min) both showed indirect sympathomimetic and, in higher concentrations, β-sympatholytic

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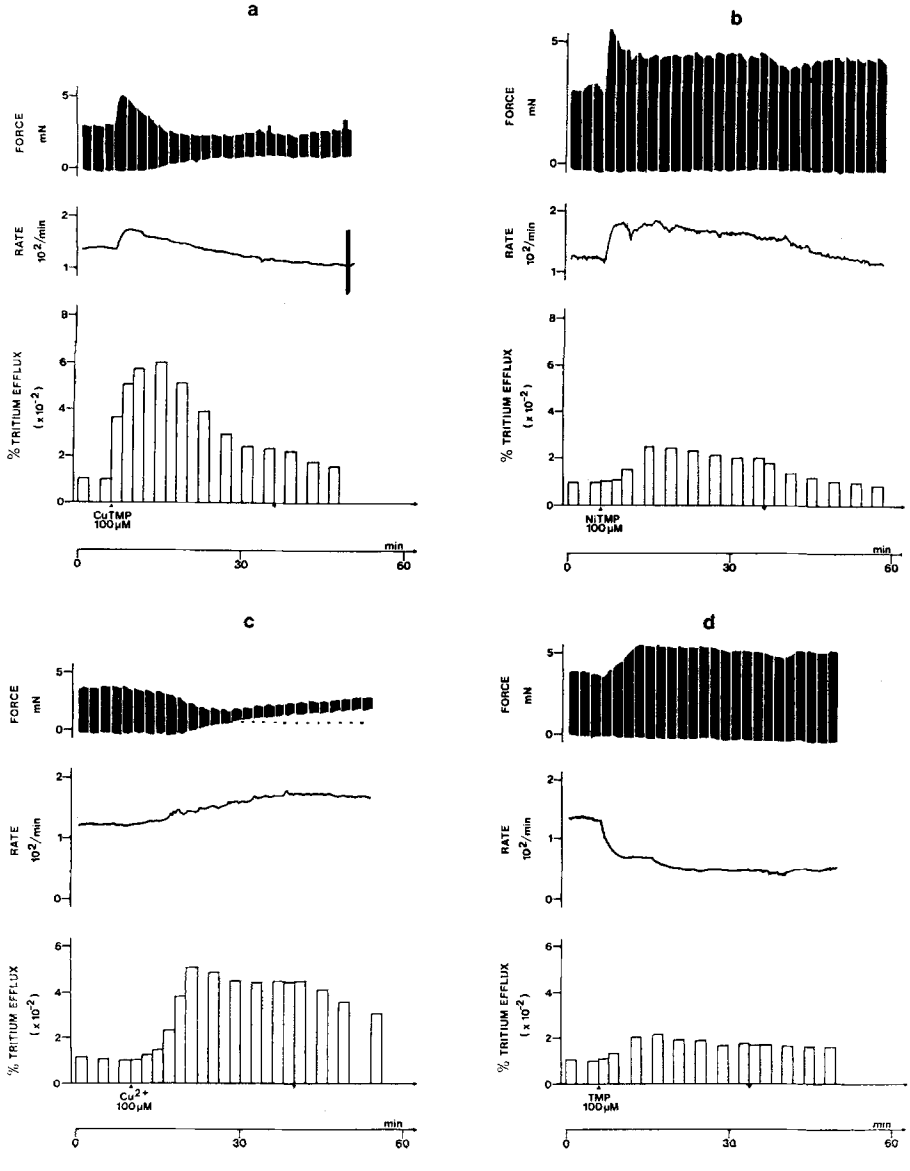

Fig. 1. The effects of the test drugs (a) CuTMP, (b) NiTMP, (c) Cu(II) ion and (d) TMP, each at 1×10^{-4}*M* for 30 min, on the force and rate of contraction of, and efflux of tritiated noradrenaline and metabolites from, the guinea-pig isolated atrium suspended in Krebs-Henseleit solution at 30 °C. Recording was stopped and the bath contents sampled for estimation of tritium efflux at two min intervals. The resting efflux of tritium before addition of test drug was in the order of 30,000 dpm/min/g of atrial wet weight (= 100 %).

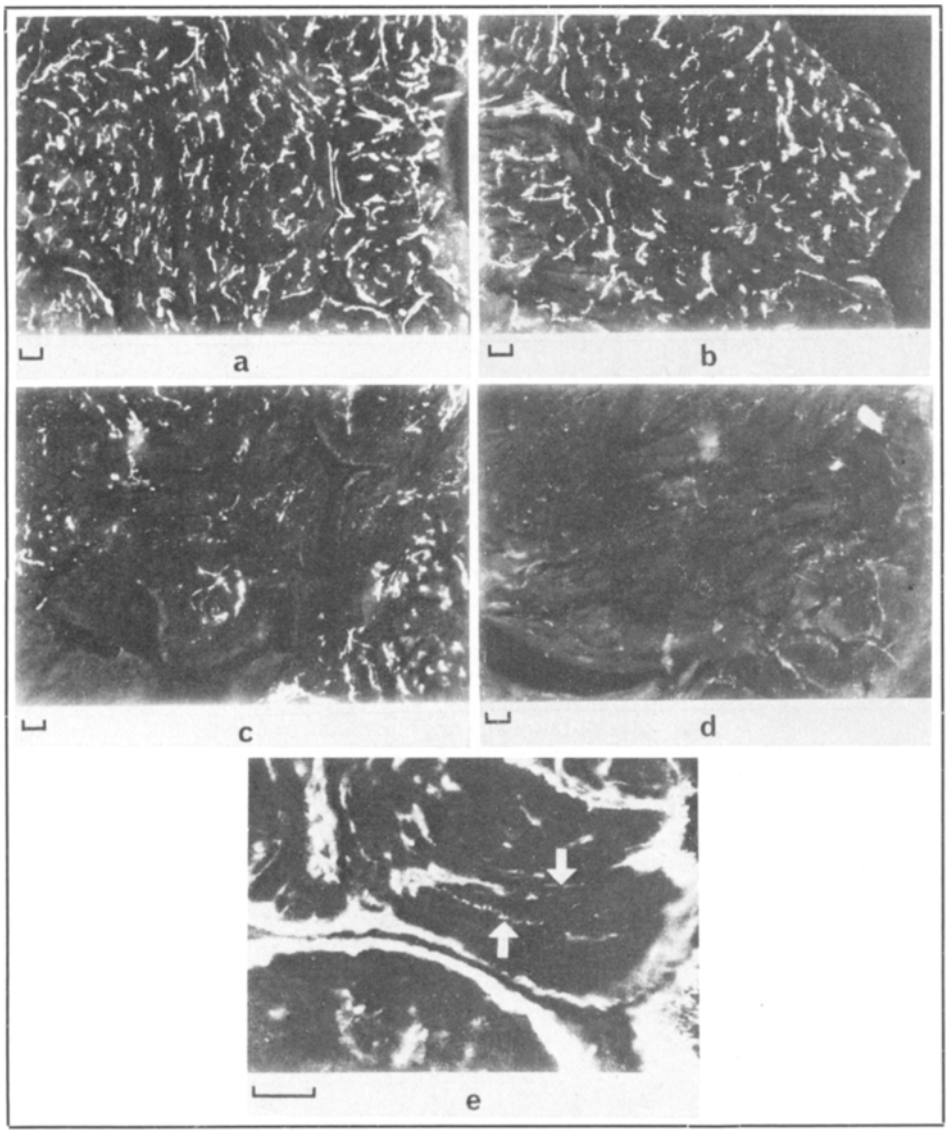

Fig. 2. Fluorescence microscopy of guinea-pig atrium following incubation alone or with test drug ($1 \times 10^{-4} M$ for 30 min) in Krebs-Henseleit solution at 30 °C. a) Control tissue, showing distribution of fluorescent adrenergic nerves. b) Tissue treated with NiTMP. No apparent diminution of the adrenergic fluorescence. c) and d) Tissue treated with CuTMP. There is patchy diminution of adrenergic fluorescence resulting both in a decreased density of nerves visualized and in a reduced intensity of fluorescence in those remaining. e) Tissue treated with RuTMP. The fluorescent chelate is bound extensively to the connective tissue surrounding individual atrial muscle bundles, and also to certain tissue elements within these bundles. Some of these are identifiable as connective tissue strands (↓). Others resemble varicose axons (↑), but most axons exhibiting adrenergic fluorescence are devoid of chelate fluorescence. Calibrations: 50 μm; excitation filter 3 mm BG 12, 530 μm barrier filter.

action which was frequently accompanied by arrhythmia and contracture of the atrial muscle; however, CuTMP was much more potent and much more rapid in onset in its action as a sympathomimetic (Figure 1). Under the same conditions, NiTMP showed weaker indirect sympathomimetic and negligible sympatholytic action, Ni(II) ion was virtually inactive and TMP increased the force of atrial contraction and the efflux of tritiated materials but produced a sustained decrease rather than an increase in the rate of atrial contraction (Figure 1). These results suggest that the active sympathomimetic species is predominantly the chelate cation as a whole rather than its constituent metal ion or ligand; a similar conclusion was reached for the action of such chelates as cholinomimetic and cholinolytic drugs[1-3] and as antimicrobial agents[2,6].

Fluorescence histochemical studies (Figure 2) showed that a 30 min exposure of the atrial preparation to CuTMP ($1 \times 10^{-4} M$) greatly decreased the intensity of the green noradrenaline fluorescence. In addition, the axonal varicosities were less pronounced than those from control tissues. Similar exposure to NiTMP, and to the corresponding, fluorescent ruthenium(II) chelate[7] of like potency, produced microscopically-detectable reduction in these parameters only at a concentration of $3 \times 10^{-4} M$. While the orange fluorescence of RuTMP was localized most strongly at surface and deeper atrial connective tissue, in some areas it also appeared to be associated with structures which resembled adrenergic varicose axons; however, most axons were devoid of chelate fluorescence.

Since the indirect sympathomimetic action of the chelates was inhibited by propranolol, reserpine, bretylium, procaine, $MgSO_4$ and a low Ca^{2+} ion concentration while cocaine and tetrodotoxin were relatively inactive, it seems likely that the primary presynaptic site of action of the fully co-ordinated divalent phenan-

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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.

H. GROSSMAN[8], A. SHULMAN[8] and C. BELL

Unit of Medical Chemistry and Department of Zoology, University of Melbourne, Parkville (Victoria 3052, Australia), 4 July 1973.

[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-

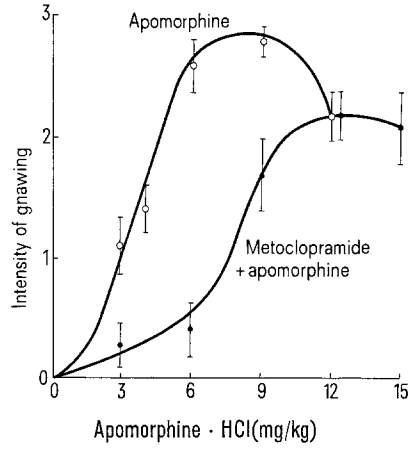

Fig. 1. Inhibition of apomorphine-gnawing by metoclopramide. Metoclopramide (0.5 mg/kg i.p.) or saline were injected 30 min before the administration of various i.p. doses of apomorphine. The gnawing compulsion was noted after a further 30 min using an intensity scale from 0 to 3. The means ± S.E. of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

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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.