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PHARMACOLOGICAL PROPERTIES OF KRYPTOPYRROLE AND ITS OXIDATION PRODUCTS ON ISOLATED SCIATIC NERVE OF RAT AND ON GUINEA-PIG ILEUM

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1 Kryptopyrrole (2, 4-dimethyl, 3-ethylpyrrole) inhibited conduction in rat sciatic nerve by a local anaesthetic action.

2 Tone and both spontaneous and electrically-induced contractions of guinea-pig ileum were also inhibited by kryptopyrrole. The concentration of kryptopyrrole required for 50% inhibition of a maximum twitch tension (ID_{50}) was 0.085 mM.

3 Oxidation products of kryptopyrrole with chromatographic properties similar to those of urinary constituents reported in schizophrenia and hepatic porphyrias had little or no effect at similar concentrations.

4 Dose-response curves to exogenous acetylcholine in guinea-pig ileum were shifted to the right by kryptopyrrole, with loss of parallelism and reduction in the maximum contraction.

5 Acetylcholine overflow from ileal segments at rest and during electrical stimulation was reduced by kryptopyrrole.

6 These results on ileal segments are consistent with kryptopyrrole having both a post-junctional site of action, presumably directly on the muscle, and a pre-junctional site reducing the output of acetylcholine from the myenteric plexus.

7 The significance of these findings is discussed in relation to a possible clinical pathological role for these compounds.

Introduction

More than 15 years ago a spot giving a purple-mauve colour with Ehrlich's reagent was reported on chromatograms of the urine of some schizophrenics (Irvine, 1961). This feature was termed 'malvaria' (Hoffer & Osmond, 1963) and it seemed possible that the unknown compound had toxic properties which were responsible for a specific disease entity, distinct from other types of mental illness. Sustained efforts over a number of years resulted in the assignment of a trialkylpyrrole structure 'kryptopyrrole' (2,4-dimethyl, 3-ethylpyrrole) to this material (Irvine, Bayne, Miyashita & Majer, 1969; Sohler, Beck & Noval, 1970). This was later revised to that of its oxidation product, 5-hydroxykryptopyrrole lactam (4-ethyl-5-hydroxy-3,5-dimethylΔ^3-pyrrolin-2-one) and still more recently to that of the corresponding oxidation product of the β-side chain isomer of kryptopyrrole, haemopyrrole (Irvine & Wilson, 1976).

Further interest in the biological activity of these compounds was stimulated by reports that they were present in the urine of patients with acute intermittent porphyria (Irvine & Wetterberg, 1972), porphyria cutanea tarda (Huszak, Durko & Karsai, 1972), hereditary coproporphyria and variegate porphyria (Brodie, Graham, Thompson, Moore & Goldberg, 1976) because in all these conditions, with the exception of porphyria cutanea tarda, peripheral neuropathy and psychiatric symptoms may be found.

Substituted pyrroles, including kryptopyrrole, have long been known to have potent toxic effects in the whole animal, including depression of the central nervous system and muscle relaxant properties (Moffet, 1968). More recent studies in mice have confirmed that kryptopyrrole has a number of behavioural effects based on general depression of the central nervous system (Wetterberg, 1973). However, little information is available on the mechanism of these effects or on the detailed pharmacological properties of kryptopyrrole and its oxidation products. It seemed of interest therefore to study these properties as a

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means of assessing a possible clinical pathological role for these compounds.

Methods

Nerve tissue

White male Wistar or brown Norwegian rats (150 to 250 g) were killed by a blow on the neck and sciatic nerves were removed and immersed in Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 1.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, and glucose 11.1, and gassed with 95% O_2 and 5% CO_2 at 37°C. Just before use the nerve trunks were blotted free of excess fluid and placed on an electrode grid set in a perspex moist chamber maintained at 37°C. The nerve was stimulated supramaximally in a proximal to distal direction with square wave pulses of 0.05 to 0.065 ms duration at 20 to 50 Hz. Action potentials were recorded from gross stainless steel external electrodes and were displayed on a Tektronix 5103N oscilloscope. Test solutions were applied directly to the nerve trunks with Pasteur pipettes or by means of small plugs of cotton wool previously immersed in the appropriate solution.

Guinea-pig ileum

Pieces of ileum, removed from animals which had fasted overnight, were suspended in the Krebs-Ringer bicarbonate-glucose solution described above but containing 1 mM $CaCl_2$ and 0.125 μM mepyramine maleate, gassed with 95% O_2 and 5% CO_2 in a 2.5 ml bath maintained at 35°C. For experiments with added histamine, mepyramine maleate was omitted. Field stimulation, 0.2 Hz, 0.5 to 1.0 ms was applied by means of platinum 'gutter' or 'ring and point' electrodes at a voltage sufficient to produce a maximum isometric twitch (80 to 100 V d.c.). The twitch tension was measured with a Statham G10B transducer and a Devices MX-2 recorder. The resting tension was set to between 0.3 and 0.75 g to yield a maximum twitch tension. Test solutions were added to the bath directly; alternatively the composition of the fluid was changed by upward displacement to a constant level outflow. Drug effects were tested 10 s after cessation of electrical stimulation.

Assay of acetylcholine

This was done essentially as described by Paton & Zar (1968), and Paton & Vizi (1969), with pieces of whole ileum, but contractions were measured with a Statham G10B transducer and a Devices MX-2 recorder.

Chemicals

Kryptopyrrole (2,4-dimethyl, 3-ethylpyrrole), lot no. 040157, was obtained from the Aldrich Chemical Co. Photo-oxidation products were prepared and purified essentially as described by Lightner & Crandall (1973). Pyrroles were suspended in Krebs-Ringer bicarbonate solution by mechanical agitation, by ultrasonic dispersion (MSE 150W ultra-sonicator), or were dispersed similarly just before use in a phospholipid sol prepared from rat hearts (cf. Gorchein, 1972). This last method was found to give the most reproducible results, probably because kryptopyrrole is insoluble in aqueous media. Porphobilinogen was isolated and crystallized from the urine of a patient with acute intermittent porphyria as described by Cookson & Rimington (1954). δ-Aminolaevulinic acid was purchased from the Sigma Chemical Co. Drugs used were acetylcholine chloride (Sigma), morphine sulphate (McCarthys), naloxone hydrochloride (Endo Labs), atropine sulphate (Antigen exports), 5-hydroxytryptamine creatine sulphate complex (Sigma), histamine acid phosphate (McCarthys), mepyramine maleate (May and Baker), and physostigmine (eserine) sulphate (Sigma).

Results

Effect of kryptopyrrole on externally recorded nerve action potentials in rat sciatic nerves

Kryptopyrrole reduced and eventually abolished the action potential with no change in conduction velocity (Figure 1). This effect was fully reversed by washing for 30 min or longer, depending on the concentration of kryptopyrrole originally applied. Action potentials recorded proximal to the site of exposure to kryptopyrrole were unaffected, indicating a local effect of the compound on the nerve trunk. The concentration of kryptopyrrole suspensions which resulted in a reduction of the action potential to 50% of its original value after a contact time of 5 min varied from 0.1 mM to 1 mM. This wide range may be due to differences in the effective concentration of kryptopyrrole in the solution-suspensions applied, and in the permeability and diffusion of the compound in the different nerve trunks, depending on their content of connective tissue. Photo-oxidized solutions of kryptopyrrole of the same original concentration had no effect on conduction. Purified components, 5-hydroxykryptopyrrole lactam, and its corresponding 5-methoxy lactam were also without effect at concentrations up to 10 mM, as were porphobilinogen and δ-aminolaevulinic acid, tested up to 2 mM.

Effects of kryptopyrrole on guinea-pig ileum

Kryptopyrrole reduced the tone of resting muscle, spontaneous activity, and the amplitude of electrically-stimulated contractions in guinea-pig ileum. Re-

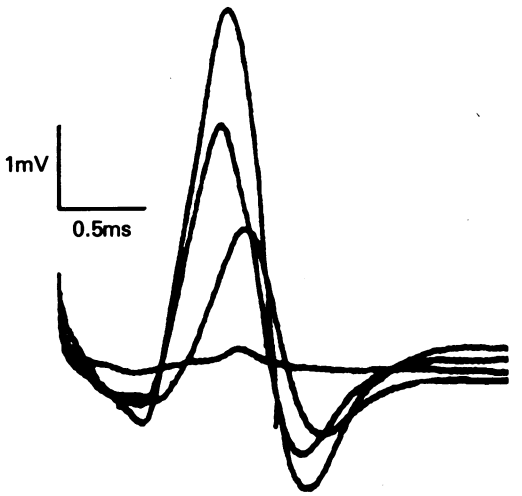

Figure 1 Effect of kryptopyrrole on rat sciatic nerve. Single sweep consecutive oscilloscope photographs of electrically-stimulated multiple compound action potentials at 5 min intervals after application of kryptopyrrole to a rat sciatic nerve trunk between the stimulating and recording electrodes. Vertical scale 1 mV, horizontal scale 0.5 ms.

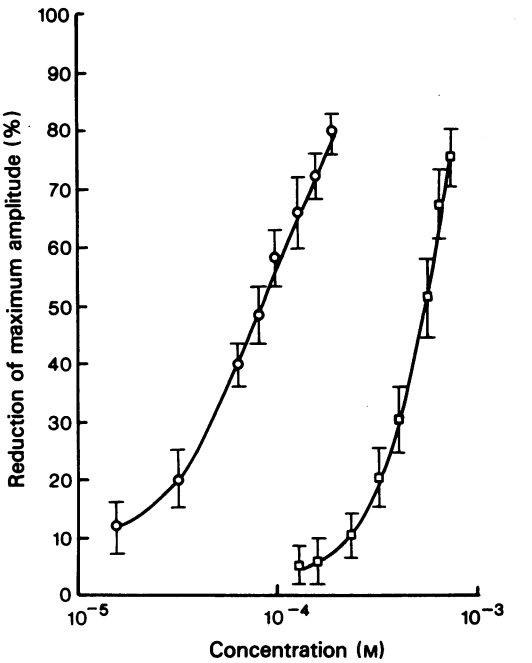

Figure 2 Dose-response curves for kryptopyrrole (○) and photo-oxygenated kryptopyrrole (□) on contractions of guinea-pig ileum to electrical stimulation. The concentration given for the photo-oxidized kryptopyrrole solution is based on its original content of intact kryptopyrrole and would therefore be less for any one of the three main components (Lightner & Crandall, 1973) produced in this reaction. Each point is the mean of 3 to 5 experiments with different pieces of ileum from different guinea-pigs; the bars are the standard errors.

sponses were determined by cumulative additions of kryptopyrrole or of its oxidation products at 5 min intervals, which was found to be long enough for the full effect of each dose to be observed. There was no tachyphylaxis and the effect was fully reversed on washing out the bath. The concentration of kryptopyrrole causing a 50% inhibition of the maximum twitch tension (ID_{50}) was 0.085 mM, (Figure 2), whilst that of photo-oxidized mixtures prepared from solutions of the same original content of kryptopyrrole was approximately 0.55 mM, representing a 6.5 fold loss of activity. Purified individual components were even less potent, 5-hydroxykryptopyrrole lactam causing only 10% inhibition at 3.5 mM. In the presence of atropine (1 μM) which completely blocked electrically-stimulated contractions, contractions mediated by 5-hydroxytryptamine (1.25 μM) were abolished by 0.2 mM kryptopyrrole, and under similar conditions there was a loss of contraction to histamine (5 μM).

Dose-response curves for exogenous acetylcholine (Figure 3) were shifted to the right with progressive loss of parallelism and reduction in the maximum response, by increasing concentrations of kryptopyrrole, consistent with non-competitive inhibition. The maximum tension developed in response to acetylcholine (0.5 μM) was 2.24 g ± 0.18 (mean ± s.e. mean of 8 experiments). The output of acetylcholine from whole segments of ileum was reduced by kryptopyrrole (Table 1). This effect was dose-dependent and most marked under resting conditions and at low rates of electrical stimulation, when up to 70% reduction was obtained. This reduction of acetylcholine output was not prevented by naloxone (80 nM).

Discussion

An inhibitory effect of kryptopyrrole on the amplitude of electrically-evoked action potentials of rat isolated sciatic nerves, with no change in conduction velocity has been shown. This inhibition was readily reversible by washing out and can thus be considered as that of a local anaesthetic. It is consistent with the development of transient ataxia *in vivo* following intraperitoneal injection of the compound in rats (unpublished experiments) and mice (Wetterberg, 1973).

In ileal segments, kryptopyrrole had both post- and pre-junctional actions. Thus the contractions produced by 5-hydroxytryptamine and histamine on the

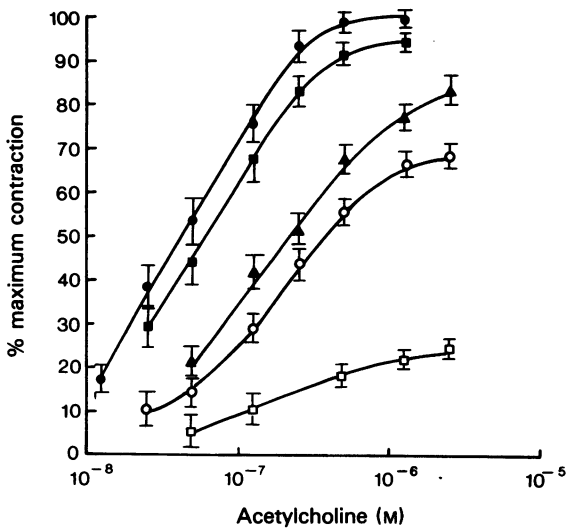

Figure 3 Effect of kryptopyrrole on dose-response curves for acetylcholine on contractions of guinea-pig ileum. Exposure to kryptopyrrole was for 3 min, which allowed the maximum reduction in amplitude to electrical stimulation to be reached. Acetylcholine was added to the bath 10 s after cessation of electrical stimulation. (●) Dose-response curve to acetylcholine in the absence of kryptopyrrole. Each point is the mean of 8 experiments on tissue from different animals. The bars are the standard errors. The other curves were obtained in the presence of concentrations of kryptopyrrole inhibiting the maximum response to electrical stimulation by (■) 10%, (▲) 30%, (○) 50% and (□) 80% ($ID_{10,30,50,80}$).

atropinized gut were inhibited by kryptopyrrole, indicating a direct inhibitory effect on the muscle. This was supported by the reduction in the maximum amplitude of contraction to exogenous acetylcholine. However, the pyrrole also reduced the output of acetylcholine from whole ileum, which probably was due to an action on its nerve plexuses, known to be the site of origin of acetylcholine in this tissue (Paton & Zar, 1968). This latter effect would be consistent also with the finding that in isolated sciatic nerve of rat, kryptopyrrole caused loss of action potentials. The site of action of kryptopyrrole on the nerve tissue of the gut is not known but it does not involve the morphine receptor since naloxone did not prevent its inhibition of acetylcholine output.

The relatively high concentrations of kryptopyrrole required for the effects demonstrated here on sciatic nerve and on gut, make it unlikely that such compounds are directly responsible for the peripheral neurological manifestations of the hepatic porphyrias. Since oxidized derivatives of kryptopyrrole were even less potent than the intact material, it would seem unlikely also that the compound reported in urine, 5-hydroxykryptopyrrole lactam or its β-side chain isomer (Irvine & Wilson, 1976) is directly responsible for peripheral neurotoxic effects *in vivo*. However, too little is known about the origin and metabolism of these substances wholly to exclude the possibility that central actions may be responsible for neuropsychiatric manifestations.

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Table 1 Effect of kryptopyrrole on acetylcholine output from guinea-pig whole ileum

Collection period (min)	*Rate of stimulation (Hz)*	*Total control acetylcholine output (pg min^{-1} mg^{-1} wet wt. ±s.e. No of expts in parentheses)*	*Concentration of kryptopyrrole in bath (μM, and ID_x)**	*% reduction of acetylcholine output*	P
20	0	48.6 ± 2.3(14)	none		
20	0	66.2(1)	35, ID_{25}	55	
20	0	46.8(2)	85, ID_{50}	62	
20	0	49.6(2)	300, ID_{90}	68	
10	0.2	79.0 ± 3.7(13)	none		
10	0.2	80.9 ± 8.8(3)	85, ID_{50}	50 ± 2.1	<0.01
10	0.2	71.5 ± 4.0(4)	300, ID_{90}	65 ± 2.2	<0.001
10	5.0	136.3(1)	300, ID_{90}	30	

* ID_x is the amount of kryptopyrrole required to inhibit the maximal contraction by x%.

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