Diameter and densities of membrane-associated particles from brain synaptosomes incubated in various media

	Diameter Å ± SD (n)		Densities particles/μm^2 ± SD (N)	
	PF-face	EF-face	PF-face	EF-face
Control (5 mM-K^+, 4 mM-Ca^{2+})	104 ± 19 (66)	99 ± 17 (21)	1145 ± 163 (11)	353 ± 58 (7)
Ca^{2+}-free (5 mM-K^+, 1 mM-EGTA)	104 ± 21 (47)	98 ± 11 (14)	1072 ± 165 (12)	397 ± 52 (10)
High-K^+ (55 mM-K^+, 4 mM-Ca^{2+})	103 ± 17 (92)	101 ± 17 (29)	1493 ± 177 (14)[a,c]	387 ± 78 (15)
High-Ca^{2+} (5 mM-K^+, 40 mM-Ca^{2+})	116 ± 20 (75)	100 ± 22 (27)	1349 ± 192 (11)[b,d]	365 ± 63 (7)

n: number of particles, N: number of fields, significantly different from control [a] $p < 0.001$, [b] $p < 0.05$, significantly different from Ca^{2+}-free, [c] $p < 0.001$, [d] $p < 0.01$.

table). Similary membrane-associated particles in the active zones of presynaptic membranes of rat spinal cord were reported to be more abundant in unanesthetized rats than in the anesthetized rats[7].

The density of particles increased on incubation of the synaptosomes in high-Ca medium. These particles also tended to be aggregated after calcium treatment (figure 2). Calcium-induced aggregation has also been observed in chromaffin granules from bovine adrenal glands[16].

These findings suggest that membrane-associated particles of the presynaptic membranes take part in neurotransmitter release in some way; for instance, as channels for ionic movement across the membrane. Further studies are required on this possibility.

1 D. Branton, Pro. nat. Acad. Sci. *55*, 1048 (1966).
2 P. Pinto da Silva and D. Branton, J. Cell Biol. *45*, 598 (1970).
3 P. Pinto da Silva and D. Branton, Chem. Phys. Lipids *8*, 265 (1972).
4 K.H. Pfenninger, K. Akert, H. Moor and C. Sandri, J. Neurocytol. *1*, 129 (1972).
5 K.H. Pfenninger and C.M. Rovainen, Brain Res. *72*, 1 (1974).
6 C. Sandri, J.M. Van Buren and K. Akert, Prog. Brain Res. *46*, 1 (1977).
7 M. Venzin, C. Sandri, K. Akert and U.R. Wyss, Brain Res. *130*, 393 (1977).
8 Y. Takano and H. Kamiya, J. Electron Microsc. *27*, 137 (1978).
9 T.W. Tillack, R.E. Scott and V.T. Marchesi, J. Cell Biol. *47*, 213 (1970).
10 P. Pinto da Silva, J. Cell Biol. *53*, 777 (1972).
11 C. Peracchia, J. Cell Biol. *57*, 66 (1973).
12 J.E. Heuser, T.S. Reese and D.M.D. Landis, J. Neurocytol. *3*, 373 (1974).
13 C. Peracchia, J. Cell Biol. *61*, 107 (1974).
14 C. Peracchia and A.F. Dulhuntry, J. Cell Biol. *70*, 419 (1976).
15 W. Duppel and G. Dahl, Biochim. biophys. Acta *426*, 408 (1976).
16 R. Schober, C. Nitsch and V. Rinne, Science *195*, 495 (1976).
17 D. Volsky and A. Loyter, Biochim. biophys. Acta *471*, 243 (1977).
18 F. Hajós, Brain Res. *93*, 485 (1975).
19 D. Branton, S. Bullivant, N.B. Gilula, M.J. Karnovsky, H. Moor, K. Mühlethaler, D.H. Northcote, L. Packer, B. Satir, P. Satir, V. Speth, L.A. Staehlin, R.L. Steere and R.S. Weinstein, Science *190*, 54 (1975).

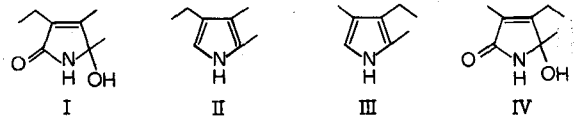
The 'mauve factor' of schizophrenia and porphyria, 5-hydroxyhaemopyrrole lactam, has low pharmacological potency on guinea-pig ileum

A. Gorchein and A.T. Rogers[1]

Department of Clinical Pharmacology and Therapeutics, St Mary's Hospital Medical School, Praed Street, London W2 (England), 16 October 1978

Summary. 5-Hydroxyhaemopyrrole lactam, the 'mauve factor' reported in the urine of schizophrenics and porphyrics was found to inhibit electrically-stimulated contractions of guinea-pig ileum only at high concentrations ($ID_{50} = 8.5$ mM). This low potency makes it unlikely that the compound can account for neurotoxic effects in human porphyria.

Reports of the excretion in urine of schizophrenics[2,3] and porphyrics[4-6] of a 'mauve factor', identified recently as the α-hydroxy-pyrrole-α'-lactam[5,7] (HPL), **I**, of haemopyrrole, 4-ethyl-2,3-dimethylpyrrole, **II**, have led us to examine some biological properties of this compound in an attempt to assess its possible clinical pathological role in these disorders. Whilst krytopyrrole, **III**, the β-side chain isomer of haemopyrrole, originally thought to be the 'mauve factor'[8,9], is highly toxic to mice and has muscle-relaxant[10] and behavioural effects[11], oxygen-free alkylpyrroles, **II** and **III**, cannot be detected in urine[12,13], and because of their high chemical reactivity with water and oxygen are unlikely to occur in other biological fluids. It has been postulated therefore that the lactams may themselves be responsible for any pharmacological actions associated with these pyrroles[5], and 5-hydroxykryptopyrrole lactam, **IV**, has been shown to increase the urinary excretion of porphyrins in rats[14] and to decrease their liver haem and cytochrome P450[15].



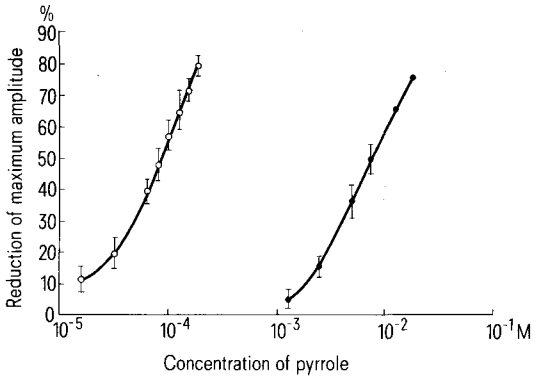
I II III IV

In previous studies[16], we compared the effects of kryptopyrrole, **III**, on nerve conduction in rat and on electrically-stimulated contraction of isolated guinea-pig ileum with those of its 5-hydroxy-lactam (KPL), **IV**. The lactam was found to have no effect on nerve conduction at up to 10 mM and to be approximately 100 times less active than its precursor in inhibiting the gut. Although these results made it unlikely that the lactam (KPL) had clinically relevant neurotoxic properties, it was felt necessary to test its β-side chain isomer (HPL) also, in case their structural differences were associated with major differences in their biological properties.

4,5-Dimethyl-3-ethyl-5-hydroxy-3-pyrrolin-2-one (HPL), **I**, was synthesized[17], purified by recrystallization and tested

on guinea-pig ileum which depends for its contraction to brief electrical stimulation on excitation of endogenous nerve tissue[18]. An inhibitory effect on electrically-stimulated contractions was apparent (figure) only at very high concentrations (ID_{50}= 8.5 mM) similar to those found previously[16] for purified KPL. It would appear extremely unlikely therefore that HPL causes peripheral neurological effects in human porphyria in which its highest reported urinary concentration was only 61 µM (9.5 mg/l)[4].

It may be that HPL is a 'detoxicated' product of a more reactive metabolite, such as haemopyrrole, which cannot itself be detected because of its lability but more quantitative and adequately controlled information about its excretion is required before diagnostic significance can be ascribed to its presence in the urine.



Dose-response curves for 5-hydroxyhaemopyrrole lactam (HPL) ● and kryptopyrrole ○ on electrically-stimulated contractions of guinea-pig ileum. Pieces of ileum were suspended in Krebs-Ringer bicarbonate-glucose solution gassed with 95% O_2 – 5% CO_2 in a 2.5 ml bath at 35 °C. Field stimulation (0.2 Hz, 0.5–1.0 msec) was applied by platinum 'ring and point' electrodes to produce a maximum isometric twitch (80–100 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 0.3–0.75 g to yield a maximum twitch tension. Responses were determined by cumulative additions of the pyrroles suspended in Krebs-Ringer solution at 5 min intervals. There was no tachyphylaxis and the effect was fully reversed on washing out the bath. Each point is the mean of 3–5 experiments with different pieces of ileum from different guinea-pigs; the bars are the SE and where none are shown, a single experiment is represented. Vertical axis: Reduction of maximum amplitude (%). Horizontal axis: Concentration of pyrrole (M).

1 We thank the Wellcome Trust for a grant.
2 D.G. Irvine, J. Neuropsychiat. *2*, 292 (1961).
3 I. Huszack, I. Durko and K. Karsai, Acta physiol. hung. *42*, 79 (1972).
4 D.G. Irvine and L. Wetterberg, Lancet *2*, 1201 (1972).
5 D.G. Irvine and D.L. Wilson, in: Porphyrins in Human Diseases p. 217, Ed. M. Doss, Karger, Basel 1976.
6 M.J. Brodie, D.J.M. Graham, G.G. Thompson, M.R. Moore and A. Goldberg, Clin. Sci. molec. Med. *50*, 431 (1976).
7 D.G. Irvine, J. Chromat. *123*, 69 (1976).
8 D.G. Irvine, W. Bayne, H. Miyashita and J.R. Majer, Nature, Lond. *224*, 811 (1969).
9 A. Sohler, R. Beck and J.A. Noval, Nature, Lond. *228*, 1318 (1970).
10 R.B. Moffet, J. med. Chem. *11*, 1251 (1968).
11 L. Wetterberg, Upsala J. med. Sci. *78*, 78 (1973).
12 S.J. Jacobson, H. Rapoport and G.L. Ellman, Biol. Psychiat. *10*, 91 (1975).
13 P.L. Gendler, H.A. Duhan and H. Rapoport, Clin. Chem. *24*, 230 (1978).
14 D.J.M. Graham, M.R. Moore, G.G. Thompson and A. Goldberg, Biochem. Soc. Trans. *4*, 1089 (1976).
15 D.J.M. Graham, G.G. Thompson, M.R. Moore, M.J. Brodie and A. Goldberg, Biochem. Soc. Trans. *5*, 1468 (1977).
16 A. Gorchein, L.D. Mitchell and A.T. Rogers, Br. J. Pharmac. *65*, 23 (1979).
17 T.A. Wooldridge and D.A. Lightner, J. heterocyclic Chem. *14*, 1283 (1977).
18 W.D.M. Paton, J. Physiol. *127*, 40 (1955).

Alteration of plasma ketamine levels in mice by probenecid

J.N. Davisson

College of Pharmacy and Health Sciences, Northeast Louisiana University, Monroe (Louisiana 71209, USA), 8 November 1978

Summary. Probenecid increases plasma ketamine levels in mice concurrently with an enhancement of duration of loss of righting reflex. The magnitude of these changes is directly related to the dose of ketamine and intervals between administration of compounds. Plasma levels of anesthetic are similar for all animals upon termination of hypnosis.

Certain pharmacological manipulations are known to alter ketamine-induced loss of righting reflex. Repeated administration of the anesthetic caused a progressive shortening of hypnosis in rats[1], and magnesium-deficient animals displayed increased sensitivities to the compound[2]. Additionally, hydroxyzine and secobarbital were shown to enhance sleeping times of humans anesthetized with this agent[3]. Conflicting reports have appeared concerning the effects of SKF-525A and phenobarbital upon ketamine induced hypnosis. One study revealed that the former caused an increase and the latter a decrease in duration of hypnosis[4], while another report indicated that these microsomal enzyme modifiers did not influence sleeping times[5].

Alterations in disposition have been offered as possible explanations for some of these observations. For example, hydroxyzine and secobarbital were shown to slow hepatic metabolism but did not alter waking plasma levels of the anesthetic[3]. However, shortening of sleeping times induced by repeated ketamine injection was suggested to result from development of an acute CNS tolerance and not to metabolic changes[1]. Self-induction of ketamine metabolism has been demonstrated, but apparently is more significant in modulating post hypnotic events[6].

Probenecid increases the duration of ketamine-mediated loss of righting reflex[7]. Since the anesthetic was shown to moderately suppress probenecid-induced accumulation of 5-hydroxyindoleacetic acid (5-HIAA) in the brain, it was suggested that lengthening of hypnosis might occur concurrently with this change. This report describes studies to determine possible relationships between probenecid-mediated prolongation of hypnosis and changes in plasma ketamine levels.

Method. Ketamine and diphenhydramine were purchased from Parke-Davis Co. (Detroit, Michigan, USA). Probene-