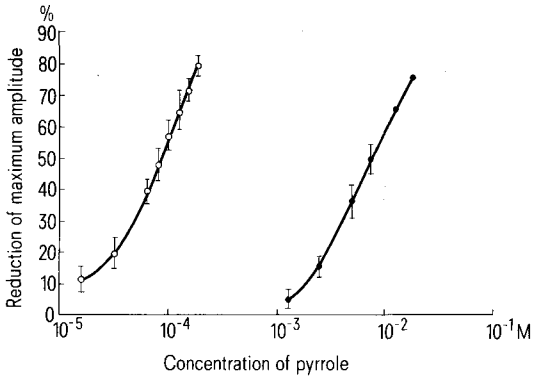
on guinea-pig ileum which depends for its contraction to brief electrical stimulation on excitation of endogenous nerve tissue[18]. An inhibitory effect on electrically-stimulated contractions was apparent (figure) only at very high concentrations (ID_{50}= 8.5 mM) similar to those found previously[16] 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).

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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-

cid solutions were prepared by dissolving the free acid, obtained by extracting commercial tablets, in a few ml of 0.2 N sodium hydroxide solution, and diluting with normal saline to produce a final concentration of 20 mg ml^{-1}. All solvents and additional chemicals were standard reagent grade materials.

Male Swiss-Webster mice (25–32 g) were provided Purina rodent laboratory chow and water ad libitum. 6 mice per cage were housed in wire meshed cages (16 × 18 × 24 cm) suspended above indirect bedding. Temperature was maintained 20–24 °C with a light period from 06.00 h to 18.00 h. All compounds were administered i.p. In addition to ketamine, all test animals received probenecid while controls were given normal saline. Approximately 2 min after ketamine injection, mice lost righting reflex. They were then placed on their backs in individual plastic cages and observed. Righting reflex was judged to be regained when an animal turned to a prone position twice within a 10-sec period. All animal experiments were conducted between 13.00 and 17.00 h.

Animals were sacrificed by decapitation, and blood collected in a small beaker. Ketamine was analyzed according to previously established methods[8].

For a normal assay procedure 0.2 ml of plasma was transferred to a glass stoppered centrifuge tube. To this was added 0.05 ml of 4 N NaOH solution, 0.05 ml of diphenhydramine hydrochloride solution (equivalent to 25 µg ml^{-1} free base), and sufficient distilled water to produce a final volume of 1.2 ml. Methylene chloride (2.0 ml) was added and the tube was shaken by hand for approximately 30 sec. Separation of the layers was facilitated by centrifugation, and the organic (lower) phase was transferred to a conical centrifuge tube. After evaporation of the methylene chloride under a stream of nitrogen (20 °C), the residue was dissolved in 0.05 ml of acetone. 1–2 µl of this solution was injected into a Perkin-Elmer 3920 B gas chromatograph equipped with a phosphorous-nitrogen (P-N) detector. The column consisted of a 180 cm × 2 mm (inner diameter) glass tube packed with 3% SP-2300 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, Pa. 16823, U.S.A.). Nitrogen carrier gas flow was maintained at 40 ml min^{-1}, hydrogen flow at 5 ml min^{-1}, and air flow at 100 ml min^{-1}. The rubidium bead current control was set at 500. Injection port and interface temperatures were 270 °C, and the column oven operated isothermally at 220 °C. Standard curves were obtained as described above except that known quantities of ketamine were added to plasma samples obtained from animals that had been injected with normal saline. Quantitation was accomplished by peak height ratio methods with diphenhydramine serving as the internal standard. The method was linear in the range 1.0–40.0 µg ketamine/sample. To confirm qualitative identification, selected samples were analyzed with a Dupont Instruments Dimaspec 321 gas chromatograph mass spectrometer interfaced with a Dupont Instruments 320 data system. Source temperature was 225 °C with ionization at 75 eV, and the accelerating voltage maintained over the range of 12,300–600 eV. The jet separator was operated at 225 °C. Other GC parameters were identical to those noted above except helium was employed as the carrier gas. Compounds were quantitated by integration of ion currents utilizing available data system software.

Results. Ketamine produced a dose dependent increase in duration of loss of righting reflex over the range of 125–200 mg kg^{-1} (table 1). This response was augmented by probenecid in animals that had received higher concentrations of the anesthetic. Additionally, at the return of righting reflex, plasma ketamine levels were essentially the same for all groups. Another series of experiments was conducted to monitor plasma ketamine concentrations over an extended time period. Following i.p. injection, levels were similar in test and control groups during the first 30 min (table 2). However, at longer time intervals, ketamine concentrations in test animals were significantly higher. Plasma levels in control and test animals after 50 and 90 min respectively, were not significantly different. This

Table 1. Influence of probenecid upon ketamine induced loss of righting reflex. Animals were treated with ketamine, followed 15 min later by probenecid (200 mg kg^{-1}) or an equivalent volume of normal saline. Plasma samples were obtained immediately after return of the righting reflex. Values represent the mean ± SEM for 10 animals

Dose[a]	Treatment	Ketamine[b]	DLRR[c]
125	Saline	9.10 ± 0.82	26.8 ± 6.9
125	Probenecid	8.75 ± 0.59	25.3 ± 4.1
175	Saline	8.95 ± 1.01	43.6 ± 4.34
175	Probenecid	9.34 ± 0.66	76.5 ± 8.59[d]
200	Saline	9.38 ± 2.39	49.7 ± 4.25
200	Probenecid	8.28 ± 0.69	91.9 ± 5.68[d]

[a] I.p. dose of ketamine (mg kg^{-1}); [b] µg ml^{-1} plasma at return of righting reflex; [c] duration of loss of righting reflex (min); [d] $p < 0.025$, according to Student's t-test, when compared to saline controls.

Table 2. Effects of probenecid upon plasma ketamine concentrations. Animals received ketamine (200 mg kg^{-1}) i.p., followed 15 min later by either probenecid (200 mg kg^{-1}) or an equivalent volume of normal saline. After indicated time periods, animals were sacrificed, and plasma collected for analysis. Values represent the mean ± SEM for 12 animals

Time[a]	Ketamine[b]
0 (S)	56.6 ± 5.70
0 (P)	53.9 ± 5.22
15 (S)	31.8 ± 2.61
15 (P)	32.2 ± 3.70
35 (S)	9.58 ± 2.09
35 (P)	22.4 ± 3.29[c]
75 (S)	1.72 ± 0.26
75 (P)	8.06 ± 0.47[c]
135 (S)	0.34 ± 0.17
135 (P)	1.68 ± 0.30[c]

[a] Min after saline (S) or probenecid (P) injection; [b] µg ml^{-1} plasma; [c] $p < 0.01$ according to Student's t-test, when compared to saline controls.

Table 3. Effect of dosing schedule upon duration of loss of righting reflex. Animals received probenecid (200 mg kg^{-1}) or an equivalent volume of normal saline 15 min prior, immediately following, or 15 min after i.p. administration of ketamine (125 mg kg^{-1}). Plasma samples were obtained upon return of righting reflex. Values represent mean ± SEM for 8 animals

Treatment	Ketamine[d]	DLRR[e]
Saline[a]	8.45 ± 0.80	27.1 ± 3.8[f]
Probenecid[a]	9.90 ± 1.20	52.4 ± 4.5
Saline[b]	8.94 ± 0.71	24.2 ± 4.8
Probenecid[b]	9.48 ± 0.59	27.1 ± 5.9
Saline[c]	9.10 ± 0.82	26.8 ± 6.9
Probenecid[c]	8.75 ± 0.59	25.3 ± 4.1

[a] Administered 15 min prior to ketamine; [b] administered immediately after ketamine; [c] administered 15 min after ketamine; [d] µg ml^{-1} plasma at return of righting reflex; [e] duration of loss of righting reflex (min); [f] $p < 0.01$, according to Student's t-test, when compared to saline controls.

correlates with other data in that these time intervals approximate average durations for loss of righting reflex (table 1).

A final group of experiments was completed to determine temporal relationships between drug administration and duration of loss of righting reflex (table 3). When injected immediately or 15 min after ketamine (125 mg kg^{-1}), probenecid caused no change. However, animals pretrated 15 min prior to receiving the anesthetic displayed significant increases in durations of loss of righting reflex. Plasma levels of ketamine were similar for all groups and in agreement with previous data (table 1).

Discussion. Results of these experiments agree with another study which showed that probenecid enhanced ketamine induced loss of righting reflex in mice[7]. Variations in doses and treatment schedules resulted in different durations of loss of righting reflex, and plasma levels of ketamine were similar for all groups of animals at the return of the righting reflex. Over time periods employed, probenecid treated mice maintained higher plasma concentrations of the anesthetic when compared to controls.

While these data suggest that probenecid causes changes in plasma ketamine concentrations, additional studies are needed to determine the underlying mechanisms. It is well documented that probenecid alters renal and biliary transport of acidic drugs, but since ketamine is basic, it would appear that competiton for transport is unlikely[9,10]. Both compounds are rapidly and extensively metabolized, with microsomal oxidation and glucuronide formation significant for each[11-13]. Since numerous agents that undergo microsomal oxidation prevent similar conversions of other compounds, competition possibly may occur at this level[3,14,15]. Also, the enhanced duration of loss of righting reflex may be related to changes in brain levels 5-HIAA[7]. Since biogenic amine metabolism was not monitored in the present study, no conclusions can be made concerning possible relationships between changes in 5-HIAA levels and plasma ketamine concentrations.

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Shape change of blood platelets induced by myelin basic protein

A. Laubscher, A. Pletscher, C.G. Honegger, J.G. Richards[1] and V. Colombo

Department of Research, Cantonal Hospital, CH-4031 Basel, and Research Division, F. Hoffmann-La Roche Inc., CH-4002 Basel (Switzerland), 5 April 1979

Summary. Myelin basic protein (MBP) isolated from bovine spinal cord caused a marked shape change reaction of human blood platelets which was not accompanied by the release reaction and not inhibited by methysergide and spiroperidol. Only those basic proteins, including MBP, which had previously shown to exert neuronal depolarisation also induced the shape change reaction. Therefore, these findings may extend the use of platelets as neuronal models.

The shape change reaction of blood platelets, characterized by a transition of their normally discoid shape into a spheroid form, can be initiated in various ways, e.g. by stimulation of the 5-hydroxy-tryptamine (5HT) receptors of the plasma membrane. These receptors have been proposed as models for the neuronal 5HT receptors in some areas of the central nervous system (CNS), since both types react in the same way to pharmacological agonists and antagonists[2]. This paper shows that myelin basic protein (MBP), a substance occurring in the CNS and causing depolarization of CNS neurons, also induces a marked shape change reaction of human blood platelets. However, this reaction is different from that caused by 5HT and may be related to MBP-induced neuronal depolarization.

Experimental. Blood platelets of healthy human volunteers were isolated by a dextran T-10 gradient, as previously described[3]. The shape change was determined with a Born Mark III aggregometer by measuring the increase in light absorption of the platelet suspensions stirred with a magnetic stirrer at 37 °C[4]. MBP, average mol.wt 18,300, isoelectric point (i.p.) > 10.6, was prepared from fresh bovine spinal cord using a slight modification of the standard method[5,6].

Furthermore, platelet suspensions stirred as indicated above and incubated for 2 min with 10^{-5} M MBP (final concentration) or with solvent (H_2O) were prefixed with an equal volume of glutaraldehyde (0.2%) and then processed for transmission and scanning electron microscopy as described earlier[3,7].

Results and discussion. Addition of MBP (in 2.5–7.5 µl H_2O) to 500 µl platelet suspension (containing 10^5 platelets μl^{-1}) caused a shape change reaction manifested by an increase in light absorption, which reached a maximum after about 1 min. The values remained maximal for 1–2 min and then decreased but were still elevated after 50 min. The EC_{50} (concentration of MBP causing half maximal effect) was $4.5 \pm 1.0 \times 10^{-7}$ M (figure 1). The 5HT content of the platelets was not changed by 10^{-6} M MBP, indicating that no marked release reaction (release of granular constituents)[8], had taken place. MBP exhibited a higher maximal