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.

1 J.F. Cumming, Anesth. Analg. *55*, 788 (1976).
2 B.G. Douglas and R. Dagirmanjian, Br. J. Anesth. *47*, 336 (1975).
3 J.N. Lo and J.F. Cumming, Anesthesiology *43*, 307 (1975).
4 M.T. Piel, J.A. Aldrette and G. Jones, Can. Anesth. Soc. J. *16*, 538 (1969).
5 M.L. Cohen and A.J. Trevor, J. Pharmac. exp. Ther. *189*, 351 (1974).
6 M.P. Marietta, P.F. White, C.R. Pudwill, W.L. Way and A.J. Trevor, J. Pharmac. exp. Ther. *196*, 536 (1975).
7 P. Ylitalo, L. Saarnivaara and L. Ahtee, Acta anaesth. scand. *20*, 216 (1976).
8 J.N. Davisson, J. Chrom. Bio. *146*, 344 (1978).
9 I.M. Weiner, J.A. Washington and G.H. Mudge, Bull. Johns Hopkins Hosp. *106*, 333 (1960).
10 L.G. Hart and L.S. Schanker, Am. J. Physiol. *211*, 643 (1966).
11 T. Chang and A.J. Glazko, in: Biodisposition of General Anesthetics, International Anesthesiology Clinics, p. 157. Little, Brown and Co., Boston 1974.
12 A.M. Guarino and L.S. Schanker, J. Pharmac. exp. Ther. *164*, 387 (1968).
13 Z.H. Israili, J.M. Perel, R.F. Cunningham, P.G. Dayton, T.F. Yu, A.G. Gutman, K.R. Long, R.C. Long, and J.H. Goldstein, J. med. Chem. *15*, 709 (1972).
14 M.W. Anders, A. Rev. Pharmac. *11*, 37 (1971).
15 A. Rubin, T.R. Tephly and G.J. Mannering, Biochem. Pharmac. *13*, 1007 (1964).
16 N.H. Neff, T.N. Tozer and B.B. Brodie, J. Pharmac. exp. Ther. *158*, 214 (1967).

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

effect than 5HT (figure 1) and strong antagonists of the 5HT-induced shape change (e.g. methysergide, spiroperidol)[2] had no effect on the action of MBP.

Electron microscopic examinations showed that platelets treated with MBP had not formed aggregates. However, they were transformed from a discoid to a spheroid form, with the extrusion of some pseudopods. There were virtually no changes in the subcellular organelles (α- and 5HT-granules) or in the microcanalicular system (figure 2), confirming that the release reaction, if present at all, was minimal.

Several basic substances, such as MBP, protamine, polyornithine and polylysine, induced both platelet shape change and neuronal depolarization, whereas spermine, spermidine and cytochrome C did not[9]. Furthermore, haloperidol (a 5HT antagonist in platelets and neurons)[2], propanolol, phenoxybenzamine and bicuculline did not markedly influence the action of MBP in either cell type[10]. Basic polypeptides, e.g. polylysine and polyornithine, have been shown to cause aggregation of platelets[11-13] and to activate other mammalian cells, including neurons[14,15], possibly by attachment of positively charged polypeptides to the negatively charged membranes. However, the effects of polypeptides do not apparently depend only on the size of their positive charge, but also their molecular weight and conformation. In fact, as the molecular weight of the basic peptide increases so does its effect on the plasma membrane of mammalian tumor cells and on platelet aggregation[14,16]. In the present experiments the shape change inducing effect (EC_{50} expressed in g/l) was also more marked with high mol.wt polyornithine (40,000) than with a low mol.wt form (4000) (Sigma, St. Louis, Mo., USA). Another basic protein, cytochrome C (mol.wt 13,000, i.p. 10.6, Merck, Germany) in contrast to MBP did not affect either platelets or neuronal cells[9].

The above findings indicate that the shape change reaction of platelets and neuronal depolarization induced by basic proteins including MBP may be initiated in a similar way. Therefore, the use of platelets as models for neurons can probably be extended by application of the shape change reaction, which under the present experimental conditions is not complicated by the release reaction or platelet aggregation[11-13,16,17]. Platelets may furnish information on

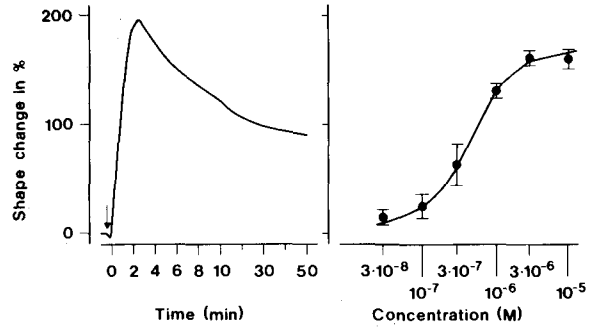

Fig. 1. Effect of myelin basic protein (MBP) on light absorption of suspensions of human blood platelets. Left: Time course; incubation with 10^{-6} M MBP (final concentration). Single experiment. Right: Effect of various concentrations of MBP after 2 min incubation. The points indicate averages with SEM of 4 experiments. On the ordinate the light absorption is indicated in percent of the maximal light absorption obtained with 5HT (10^{-5} M, after 1 min).

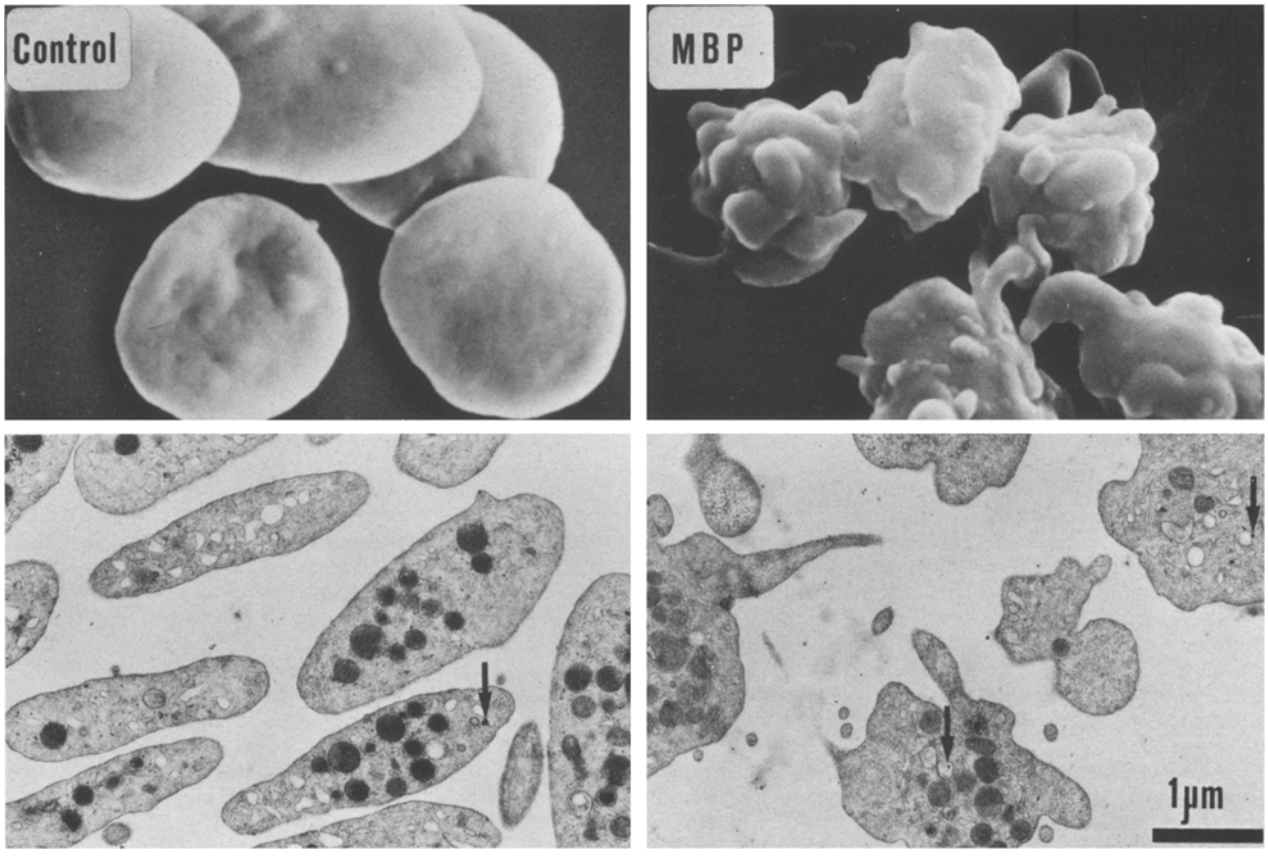

Fig. 2. Scanning (above) and transmission (below) electron micrographs of normal (left) and MBP-treated (right) platelets. Arrows: 5HT organelles; these are rather rare in human platelets and often only partially filled with osmiophilic material (5HT).

the mode of action of substances causing neuronal depolarization. For instance, ouabaine, an inhibitor of Na^+-K^+-ATPase[18] in contrast to MBP, did not cause any shape change in platelets. This indicates that the MBP-induced shape change cannot be due to inhibition of Na^+-K^+-ATPase, and that the depolarizing effects of ouabaine[9] and of MBP in neuronal cells may well have different natures. Platelets might also be used to screen for substances able to antagonize the effects of MBP on membranes, which would be of potential interest for demyelinating diseases e.g. multiple sclerosis. In these disorders proteins seem to be liberated from the myelin sheaths, whose content of MBP, but not of histones, is quite high[19]. Indeed, increased amounts of MBP-like material have been found in the cerebrospinal fluid of patients with demyelinating disorders[20-22]. If these proteins were to act on neuronal membranes this might cause neurological manifestations e.g. during episodes of acute exacerbation of the disease. Finally, it may be of interest to investigate whether there is a difference between platelets of patients with demyelinating disorders and those from healthy controls in their reaction to MBP.

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2 M. Graf and A. Pletscher, Br. J. Pharmac., in press (1979).
3 M. Graf, A. Laubscher, J.G. Richards and A. Pletscher, J. clin. Lab. Invest., in press (1979).
4 F. Michal and G. Born, Nature *231*, 220 (1971).
5 G.E. Deibler, R.E. Martenson and M.W. Kies, Prep. Biochem. *2*, 139 (1972).
6 P.R. Dunkley and P.R. Carnegie, in: Research Methods in Neurochemistry, vol. 2, p. 219. Ed. N. Marks and R. Rodnight. Plenum Press, New York 1974.
7 J.C. Mattson, P.J. Borgerding and D.L. Crafft, Stain Technol. *52*, 151 (1977).
8 H.J. Weiss, New Engl. J. Med. *293*, 531 (1975).
9 B.H. Gähwiler and C.G. Honegger, Neurosci. Lett. *11*, 317 (1979).
10 C.G. Honegger, B.H. Gähwiler and H. Isler, Neurosci. Lett. *4*, 303 (1977).
11 K.A. Gröttum, Thromb. Diath. haemorrh. *21*, 450 (1969).
12 C.S.P. Jankins, M.A. Packham, R.L. Kinlough-Rathbone and J.F. Mustard, Blood *37*, 395 (1971).
13 J.G. White, Am. J. Path. *68*, 447 (1972).
14 H.J.-P. Ryser, Science *159*, 390 (1968).
15 R.T. Hadley and C. Trachtenberg, Brain Res. *158*, 1 (1978).
16 Y. Taketomi and A. Kuramoto, Thrombos. Haemostas. *40*, 11 (1978).
17 P. Massini, L.C. Metcalf, U. Näf and E.F. Lüscher, Haemostasis *3*, 8 (1974).
18 J.L. Moake, K. Ahmed, N.R. Bachur and D.E. Gutfreund, Biochim. biophys. Acta *211*, 337 (1970).
19 P.E. Braun and S.W. Brostoff, in: Myelin, p. 201. Ed. P. Morell. Plenum Press, New York 1977.
20 S.R. Cohen, R.M. Herndon and G.M. McKhann, New Engl. J. Med. *295*, 1455 (1976).
21 S.R. Cohen and H.S. Gutstein, Science *199*, 301 (1978).
22 J.H. Carson, E. Barbarese, P.E. Braun and T.A. McPherson, Proc. nat. Acad. Sci. USA *75*, 1976 (1978).

Effect of the polychlorinated biphenyl preparation, Aroclor 1242, on the quantity of neurosecretory material in the medulla terminalis X-organ of the fiddler crab, *Uca pugilator*[1]

R. Nagabhushanam, S.W. Fingerman and M. Fingerman[2]

Department of Zoology, Marathwada University, Aurangabad (India) and Department of Biology, Tulane University, New Orleans (Louisiana 70118, USA), 20 October 1978

Summary. Exposure of the fiddler crab, *Uca pugilator*, to the PCB preparation, Aroclor 1242, produces an increase in the quantity of neurosecretory material in the medulla terminalis X-organ. This Aroclor apparently inhibits release, but not synthesis, of one or more neurohormones.

Polychlorinated biphenyls (PCBs) are extremely persistent, globally distributed pollutants[3]. Many animals, including the fiddler crab, *Uca pugilator*, the species used in this investigation, have been found to accumulate PCBs in their tissues[4]. Exposure of this crab to the PCB preparation, Aroclor 1242, results in decreased dispersion of the pigment in its melanophores[5]. Those crabs exposed to Aroclor 1242 had up to 4 times more melanin-dispersing hormone (MDH), a neurohormone, in their eyestalks than did control crabs. The PCB preparation appeared to inhibit MDH release but not its synthesis. In the eyestalk of the fiddler crab, MDH is released from the sinus glands. These glands are neurohemal organs. Most, if not all, of the neurosecretory axons that enter a sinus gland originate in the medulla terminalis X-organ of each eyestalk. In a recent study of the eyestalk of the crayfish, *Orconectes virilis*, the investigators could find no evidence that neurosecretory axons came from any other area than the medulla terminalis X-organ[6]. The object of this investigation was to determine whether cytological evidence of an effect of Aroclor 1242 on the neuroendocrine system of the fiddler crab could be obtained.

Materials and methods. Mature female specimens of *Uca pugilator*, having a carapace width of 1.5–1.6 cm, from the area of Panacea, Florida, were used. At noon, crabs were selected from the stock supply and placed in white enameled pans (18 cm diameter) containing artificial sea water (Instant Ocean, Aquarium Systems) alone, and placed under a constant illumination of 2100 lux at 24 °C. 24 h later those crabs whose melanophores were at Hogben-Slome stage 3 were selected for further use[7]. According to the Hogben-Slome scheme stage 3 represents an intermediate degree of pigment dispersion with stage 1 representing maximal concentration of the pigment and stage 5 maximal dispersion. The selected crabs were then divided into 2 groups and placed into white enameled pans which contained either 8 ppm Aroclor 1242 (Monsanto Lot Number G266K) in a solution of 0.1% acetone in artificial sea water or only 0.1% acetone in artificial sea water. The Aroclor had first been dissolved in acetone which explains the presence of acetone in both the experimental and control containers. The volume of liquid in each pan was 400 ml. The 2 groups of crabs were again exposed to the constant illumination of 2100 lux at 24 °C for 24 h after which their melanophore stages were again determined and their eyestalks were removed. The internal tissues of both eyestalks from 5 experimental and 5 control crabs were then dissected out, fixed in Bouin's solution, embedded in paraffin, cut into longitudinal serial sections 8 µm thick, and stained with Gomori's chrome-alum hematoxylin and