Liberation of Platelet Histamine by the Alkaline Tissue Extract and Oleic Acid

Although it has been well known that certain alkaline tissue extracts have an ability to cause release of 5-hydroxytryptamine (5-HT) from the platelets[1-4], characteristics of this active principles have been elucidated only recently, explaining that the fraction of unsaturated fatty acids separated from the extracts is responsible for the 5-HT releasing action[5-7]. This was confirmed by using oleic or linoleic acid instead of the extracts. The work described in the present paper extends the earlier investigations to see whether platelet histamine can also be affected by these extracts or oleic acid.

Experimental. Rabbits (both sexes, 1.8–2.3 kg) were bled through a polyethylene cannula from the carotid artery. Blood was collected in a siliconized flask containing 1% disodium ethylene diamine tetraacetate in 0.9% sodium chloride in a volume equal to $^1/_{10}$ of the total. Platelets were isolated as previously described[5].

For preparation of the 5-HT releasing components in the tissue extracts, rabbit kidney was mainly used. After alkaline treatment[1], extraction with 70% (v/v) ethanol followed by dry acetone was carried out. The fraction of mono- and di-enoic acids separated on a thin layer chromatography[5] was used for the experimental material. Oleic acid used was a commercial product, its purity being over 98%.

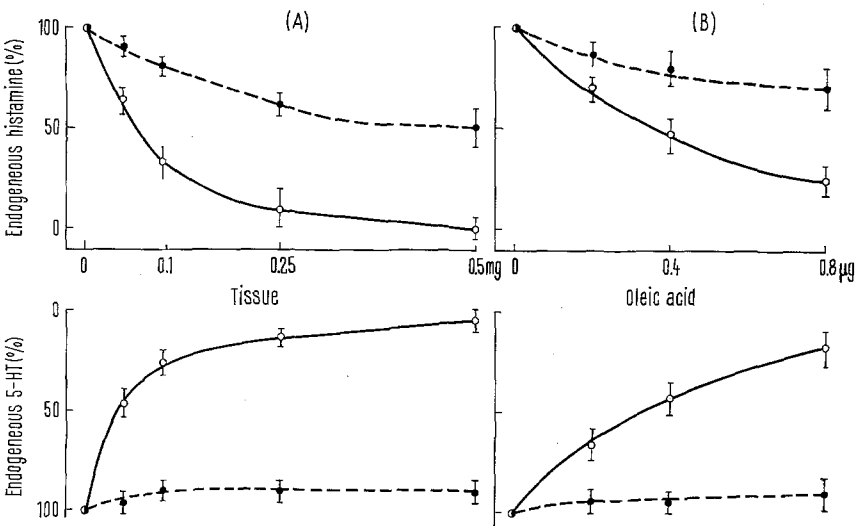
10^7–10^8 platelets were suspended in a gelatine-omitted Tullis-Toh's solution[5] containing test materials when required. Volume of the mixture was adjusted to 5 ml and incubated aerobically at 37 °C for 60 min. At the end of incubation period, the platelets were separated by centrifugation at 1000*g* for 30 min, and their histamine (and in some cases, spermidine) content was measured fluorophotometrically[8]. Estimation of 5-HT content was carried out as described previously[5].

Results. 1. As described earlier[5], rabbit platelets lost (approximately 10% of the original content) 5-HT during 60 min of incubation at 37 °C, but the rate of the spontaneous release of histamine was higher, reaching an extent of 20–30% in the present experimental conditions, while hardly any loss was observed at 0 °C. In the presence of glucose, however, such a leakage of histamine was not prevented. Trace amounts of spermidine were detected in the intact platelets.

2. When the platelets were incubated with the alkaline tissue extracts, marked liberation of histamine into the incubation medium was clearly observed. As shown in Figure 1, A, the amount of 0.25 mg tissue equivalent was enough to produce almost complete liberation of the platelet histamine (upper half of the figure). In contrast, the platelets became insensitive to the extracts when (1 m*M*) glucose was supplemented in the incubation medium. A mirror image can be recognized from the figure in which such histamine release and glucose interaction were very similar to the case of 5-HT (lower half of the figure). Total recovery of histamine, sum of the amount of platelet-retained and -released amine, was lowered as the extent of histamine release proceeded, and, when release of 90% or more was attained, only 80 to 85% of histamine could be recovered. These observations suggested that the platelet cytoplasm and/or membrane contained an active histamine metabolizing enzyme which could break down free histamine, but not histamine still contained in the platelet granules[9,10].

3. As already reported, active components in the alkaline tissue extracts were identified to be certain

[1] C. C. Toh, J. Physiol., Lond. *133*, 402 (1956).
[2] C. C. Toh, J. Physiol., Lond. *138*, 488 (1957).
[3] N. J. Giarman, L. T. Potter and M. Day, Experientia *16*, 492 (1960).
[4] A. B. Elliott, J. Physiol., Lond. *165*, 83 (1963).
[5] H. Shio, Naunyn-Schmiedebergs Arch. Pharmak. *264*, 147 (1969).
[6] A. Inouye, H. Shio, M. Sorimachi and K. Kataoka, Experientia *26*, 308 (1970).
[7] H. Shio, Naunyn-Schmiedebergs Arch. Pharmak., *267*, 123 (1970).
[8] I. A. Michaelson and P. Z. Coffman, Biochem. Pharmac. *16*, 2085 (1967).
[9] M. Da Prada, A. Pletscher, J. P. Tranzer and H. Knuchel, Nature, Lond. *216*, 1315 (1967).
[10] A. Pletscher, Br. J. Pharmac. Chemother. *32*, 1 (1968).



Effect of various concentrations of the alkaline tissue extracts (A) and oleic acid (B) on the histamine (upper) and 5-HT (lower) content of rabbit platelets. Incubations were carried out at 37 °C for 60 min. Dotted lines indicate the results obtained in the presence of glucose. Each point represents an average with S.E. (vertical bars) of 3 to 5 experiments. 10^7 platelets contained 0.5 ± 0.1 µg histamine and 1.6 ± 0.2 µg 5-HT before incubation.

unsaturated fatty acids[5–7]. In the present experiments also this was clearly confirmed as shown in Figure 1, B. When oleic acid was substituted for the tissue extracts, the liberation pattern of histamine and 5-HT has a strong resemblance to that of the tissue extracts.

Discussion. According to the present results, certain unsaturated fatty acids affect platelets to liberate not only 5-HT but also histamine. Several cytochemical and electronmicroscopical studies have indicated that platelet histamine is contained in bound state in their storage granules as 5-HT[9–13]. Further, our electronmicroscopical observations have demonstrated typical degranulation of rabbit platelets after incubation with the alkaline tissue extracts (to be published). It is highly probable, therefore, that certain unsaturated fatty acids first affect amine-containing granules in platelets which in turn cause release of 5-HT and histamine from the platelets. Similar observations have already been made in this laboratory on the nucleated thrombocytes[14–16].

Zusammenfassung. Durch In-vitro-Inkubation mit alkalischen Gewebsextrakten oder mit Oleinsäure wurde Histamin aus den Blutplättchen von Kaninchen freigesetzt.

K. KATAOKA, M. SORIMACHI, H. SHIO, S. NAGATA and A. INOUYE

Department of Physiology, Kyoto University School of Medicine, 606 Kyoto (Japan), 5 June 1970.

[11] I. J. BAK, R. HASSLER, B. MAY and E. WESTERMANN, Life Sci. *6*, 1133 (1967).
[12] M. DA PRADA, A. PLETSCHER, J. P. TRANZER and H. KNUCHEL, Helv. physiol. pharmac. Acta *25*, 430 (1967).
[13] M. DA PRADA and A. PLETSCHER, Br. J. Pharmac. Chemother. *34*, 591 (1968).
[14] A. INOUYE, K. KATAOKA, M. SORIMACHI and S. HORI, Europ. J. Pharmac. *8*, 200 (1969).
[15] M. SORIMACHI, K. KATAOKA, A. INOUYE and S. HORI, Europ. J. Pharmac., in press (1970).
[16] I. KURUMA, F. OKADA, K. KATAOKA and M. SORIMACHI, Z. Zellforsch., *108*, 268 (1970).

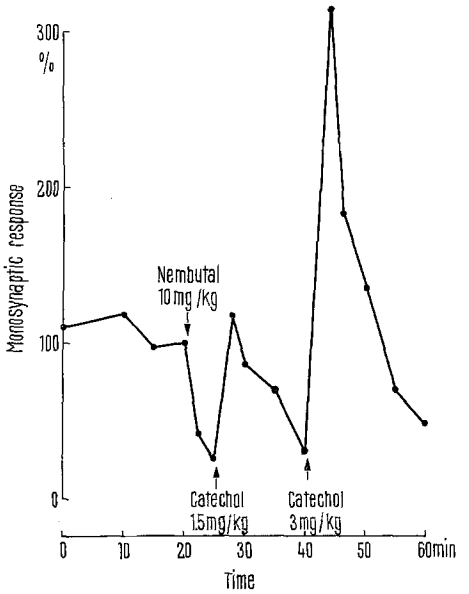
Antagonism of Barbiturate Depression of Spinal Transmission by Catechol

It is generally believed that the synapse is the major site of action of barbiturates at all levels of the neuraxis. In the spinal cord, barbiturates depress transmission across both monosynaptic and polysynaptic reflex arcs[1]. Recent neurophysiological investigations have probed into the possible mechanism of this synaptic depression. LØYNING et al.[2] have observed a decrease in potentials which represent activity in spinal afferent nerve terminals after barbiturate administration, while the firing threshold of the motoneuron remained constant. They postulated that barbiturates act mainly on these terminals resulting in decreased transmitter release and leading to reduction of synaptic potentials. Subsequently, WEAKLY[3] showed that an interference with the mechanism responsible for transmitter release could account entirely for the barbiturate-induced depression of transmission in the spinal monosynaptic pathway.

Recent work on convulsant phenols[4] has demonstrated the ability of these compounds to facilitate central synaptic transmission and produce spinal actions opposite to those reported for barbiturates. The observed facilitation induced by phenolic substances was postulated to result mainly from increased transmitter release by afferent presynaptic terminals after nerve stimulation. Phenolic substances may therefore be expected to antagonize the effects of barbiturates on synaptic transmission.

Cats (2.5–4.2 kg) were anesthetized with ether, then spinalized by a section at the atlanto-occipital junction. Ether anesthesia was terminated and artificial respiration instituted and maintained during the experiment. Carotid arteries were ligated. The lumbosacral cord was exposed by laminectomy, the dura sectioned, and all nerve roots from L_5 to S_3 were severed extradurally on one side. On the other side, L_5, S_2 and S_3 nerve roots were cut, along with the ventral roots of L_6, L_7 and S_1. The spinal cord was bathed in mineral oil at a constant temperature of 37 °C. Body temperature was also maintained close to 37 °C.

Square wave stimuli of 0.1 msec duration maximal for group I fibers were applied to the hamstring nerve and the evoked potentials measured from ventral L_7. Stimulating electrodes were also placed on the sural nerve, while all the other leg nerves were cut. Exposed nerves were covered with warm mineral oil. A filament from dorsal L_6 was used to record the dorsal root reflex. Occasionally the cut L_7 dorsal root was stimulated supramaximally and the evoked discharge recorded from L_7 ventral root.



Typical experiment showing the antagonism of barbiturate-induced depression of the spinal monosynaptic response by catechol. The hamstring nerve was stimulated and the evoked ventral root discharge was recorded from the ipsilateral L_7 ventral root. Drugs were administered i.v.

[1] A. WIKLER, Proc. Soc. exp. Biol. Med. *58*, 193 (1945).
[2] Y. LØYNING, T. OSHIMA and T. YOKOTA, J. Neurophysiol. *27*, 408 (1964).
[3] J. N. WEAKLY, J. Physiol. *204*, 63 (1969).
[4] N. R. BANNA and S. J. JABBUR, Brain Res. *20*, 471 (1970).