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Release of endogenous noradrenaline and 5-hydroxytryptamine from human blood platelets by thrombin

ALFRED PLETSCHER, *Department of Research, University Clinics, Kantonsspital, 4031 Basel, Switzerland.*

In human platelets exogenous 5-hydroxytryptamine (5-HT) and probably also noradrenaline (NA) are localized in specific subcellular organelles, the dense bodies. Indeed, thrombin which causes exocytosis of the contents of these organelles (Reimers et al 1976) markedly decreased the levels of [^{14}C]5-HT and [^{3}H]-NA in platelets preincubated with these amines (Peyer & Pletscher 1981). Endogenous 5-HT also shows a preferential localization in the dense bodies (Da Prada et al 1972). However, in human platelets the subcellular distribution of endogenous NA, whose concentration is about 3 orders of magnitude lower than that of 5-HT (Da Prada & Picotti 1979), has not yet been established. Therefore, it cannot be excluded that a relatively large proportion of the endogenous NA is located in an extragranular pool, not releasable by thrombin.

This paper shows that in human platelets thrombin causes not only a marked and rapid release of endogenous 5-HT, but also of endogenous NA.

Blood platelets from healthy volunteers were isolated as previously described (Graf et al 1979) using a discontinuous gradient of dextran dissolved in Tris-buffer containing 0·38% (w/v) trisodium citrate (TSC). Samples removed from the gradient (0·5 ml, with an approximate concentration of 10^9 platelets ml^{-1}) were incubated in polycarbonate tubes at 37°C with $CaCl_2$ (final concentration 3×10^{-3} M) and various amounts of thrombin of specific activity of 55 international units (i.u.) per mg protein. The thrombin was kindly provided by Dr R. Strässle, F. Hoffmann-La Roche Inc. Basel/Switzerland. For the experiments with imipramine, the platelet suspension (0·9 ml, final concentration approximately 3×10^8 ml^{-1}) was preincubated in Tris at 37°C for 20 min with or without 2×10^{-7} M (final concentration) of the drug. Creatinine phosphate (10 mM) and creatinine phosphokinase (40 µl ml^{-1}) were also added to the preincubation mixture, to transform released adenosine-3′,5′-diphosphate into the triphosphate. Afterwards the mixture was supplemented with thrombin plus 3×10^{-3} M (final concentration) $CaCl_2$ in 0·1 ml aqueous NaCl (0·9%) and the experiment continued as above. These conditions were found to be optimal for demonstrating that the reuptake of endogenous 5-HT was imipramine-sensitive. The incubations were stopped after various times by cooling the tubes in ice-water, then the platelets were isolated by centrifugation and washed once with Tris-TSC. The whole procedure was carried out at 4°C if not otherwise stated. The platelets from five incubation samples from the same donor were pooled and the contents of endogenous NA and 5-HT in the platelets were measured by a radioenzymatic (Da Prada & Zürcher 1976) and a spectrophotofluorimetric (Shellenberger & Gordon 1971) method respectively. The amine concentration was calculated in each case as a percentage of that present before incubation with thrombin. In the imipramine experiments the 5-HT-values after the preincubation without the drug were taken as controls. These values were virtually identical with those after preincubation with imipramine.

Fig. 1a shows that 0·3 i.u. thrombin caused a rapid decline in endogenous 5-HT and NA in the platelets, indicating that the amines have been released. The decrease of 5-HT, but not that of NA, was followed by a partial recovery. The diminutions of both NA and 5-HT were dependent on the concentration of thrombin, the decline of NA being more marked than that of 5-HT (Fig. 1b). The partial recovery of 5-HT as well as the less marked effect of thrombin on 5-HT than on NA are explained by reuptake of 5-HT after its liberation, due to the existence at the plasma membrane of an efficient uptake mechanism for 5-HT but not for NA (Pletscher 1978). This view is confirmed by the results of our experiments with imipramine. In fact, in the presence of this inhibitor of 5-HT-uptake (2×10^{-7} M) 0·3 i.u. thrombin caused a decrease of the 5-HT-level of platelets from $4{\cdot}42 \pm 1{\cdot}03$ nmols/10^9 platelets (controls) to $0{\cdot}59 \pm 16/10^9$ after 1 min, and the amine showed practically the same value ($0{\cdot}58 \pm 0{\cdot}13$) at 30 min. In platelets not incubated with imipramine thrombin diminished the 5-HT-content to $1{\cdot}03 \pm 0{\cdot}23$ nmol/10^9 after 1 min and after 30 min the 5-HT had partly recovered ($1{\cdot}45 \pm 0{\cdot}31$; $P<0{\cdot}01$) (Student's two-tailed t-test; n = 5 in each experiment). Therefore, the action of thrombin on

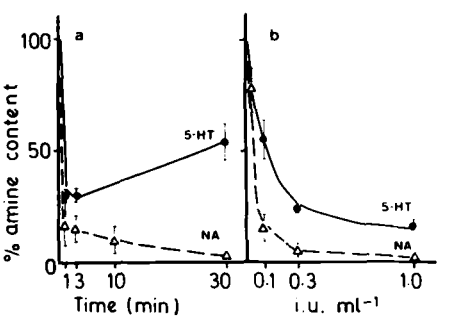

FIG. 1. Effect of thrombin (in the presence of 3×10^{-3} $CaCl_2$) on the contents of endogenous 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in human platelets. The amine content of the platelets before incubation (0 min) was taken as 100% in each experiment. a: incubation with 0·3 international units (i.u.) of thrombin, b: incubation for 3 min. Each point is an average with s.e.m. of 4–5 (NA) and 3–6 (5-HT) experiments.

endogenous 5-HT and NA was similar to that described before on the exogenous amines (Reimers et al 1976; Peyer & Pletscher 1981).

The present finding that most of the endogenous NA like the 5-HT is releasable by thrombin indicates a preferential localization of endogenous NA in the granular pool of human platelets. Therefore, when the release reaction occurs in vivo, e.g. in the course of platelet aggregation, NA is likely to be liberated together with 5-HT. Although the absolute amounts of liberated NA can only be very small (NA-content in our experiments: $4{\cdot}67 \pm 0{\cdot}75$ pmol/10^9 platelets), some local action of this amine (released, for instance, from platelet clots in microvessels) cannot be excluded. This would be especially the case when the platelets contain elevated amounts of NA, as in situations of stress (Da Prada & Picotti 1979). A further factor to be considered is that the released NA unlike 5-HT cannot be inactivated by reuptake into the platelets.

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REFERENCES

Da Prada, M., Picotti, G. B. (1979) Br. J. Pharmacol. 65: 653–662

Da Prada, M., Zürcher, G. (1976) Life Sci. 19: 1161–1174

Da Prada, M., Tranzer, J. P., Pletscher, A. (1972) Experientia 28: 1328–1329

Graf, M., Laubscher, A., Richards, J. G., Pletscher, A. (1979) J. Lab. Clin. Med. 93: 257–265

Peyer, M., Pletscher, A. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 316: 81–86

Pletscher, A. (1978) in: Youdim, M. B. H. (ed.) Essays in neurochemistry and neuropharmacology, vol. 3. John Wiley & Sons, Ltd., London, pp 49–101

Reimers, H. J., Kinlough-Rathbone, R. L., Cazenave, J.-P., Senyi, A. F., Hirsch, J., Packham, M. A., Mustard, J. F. (1976) Thromb. Haemostas. 35: 151–166

Shellenberger, M. K., Gordon, J. H. (1971) Anal. Biochem. 39: 356–372

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Effect of trazodone, mianserin, iprindole and zimelidine on wet dog shakes produced by carbachol in rats

L. Turski*, S. J. Czuczwar, W. Turski, Z. Kleinrok, *Department of Pharmacology Institute of Clinical Pathology, Medical School, Jaczewskiego 8, PL 20–090 Lublin, Poland*

Intracerebroventricular administration of a potent cholinomimetic agent, carbachol chloride, produced wet dog shakes in rats (Turski et al 1981a). Evidence derived from our behavioural and biochemical studies, suggests that this effect may be interpreted in terms of the unique imbalance, between the central cholinergic and noradrenergic activity (unpublished data) and it seems to be independent of 5-hydroxytryptaminergic functions (Turski et al 1981b). Recently, we have reported that the shaking behaviour apparently responds to treatment with tricyclic antidepressants and monoamine oxidase inhibitors (Turski et al 1981c). Thus, the question arises of the possible use of carbachol-induced behavioural responses as an animal model sensitive to antidepressant treatment. Trazodone, mianserin, iprindole and zimelidine have been found to be effective in the treatment of depression (Daneman 1967; Brogden et al 1978; Ayd 1979; Montgomery 1980), although their mechanism of action still remains unclear. Although these drugs differed widely in their profiles of action, we attempted to study their influence on carbachol-induced wet dog shakes.

Male Wistar rats (180–210 g) were treated with intraperitoneal injections of 0·9% NaCl (saline), trazodone . HCl (2·5, 5, 10, 20 mg kg^{-1}; Francesco Angelini, Rome, Italy), mianserin HCl (5, 10, 15, 20 mg kg^{-1}; Organon, West Orange, N.J., U.S.A.), iprindole HCl (1, 2, 4, 8 mg kg^{-1}; Wyeth, Windsor, Ontario, Canada) or zimelidine HCl (2·5, 5, 10, 20 mg kg^{-1}; Astram Södertälje, Sweden), all antidepressants being administered in saline 0·5 ml/100 g 1 h before intracerebroventricular (i.c.v.) administration of carbachol chloride (OY Star AB, Tampere, Finland: 10 μg), dissolved in 10 μl sterile buffered saline, pH = 7·35 according to Herman (1970).

* Correspondence.

Eight rats, assigned to all experiments by means of a completely randomized schedule, were put into individual Plexiglas cages (25 × 15 × 10 cm) placed in a well-lighted and quiet room maintained at 21 ° ± 1 °C. Wet dog shakes were counted immediately after i.c.v. carbachol chloride over a duration of 60 min. Saline-injected i.p. or i.c.v. rats served as respective controls. The data collected from behavioural experiments were treated by means of Student's *t*-test.

Fig. 1 shows the effect of drugs on carbachol-induced wet dog shakes. Trazodone dose-dependently antagonized the response while mianserin induced a statistically significant reduction only at the dose of 20 mg kg^{-1}. Significant potency against the behaviour was displayed by iprindole while zimelidine (20 mg kg^{-1}) only slightly modified it. The numbers of wet dog shakes in the control rats were less than 1 during the 60 min.

There is no evidence so far that trazodone, mianserin, zimelidine or iprindole may have potent anti-acetylcholine activity (Leonard 1980), so the efficacy of these drugs against carbachol-induced wet dog shakes cannot be ascribed to the blockade of central muscarinic cholinergic receptors. The anti-5-HT properties of trazodone and