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A simple superfusion technique for studying release of radiolabelled 5-hydroxytryptamine from blood platelets without interference of reuptake

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Blood platelets have been suggested as an experimental model for monoamine-containing nerve endings (Sneddon, 1973). For instance, *in vitro* these organelles accumulate 5-hydroxytryptamine (5-HT) against a concentration gradient by a temperature- and sodium-dependent process, which can be described by Michaelis-Menten kinetics (Sneddon, 1969), similar to the active (re)uptake mechanisms for several neurotransmitters in nerve terminals (Iversen, 1970; Snyder, Kuhar & others, 1970). Also, with regard to the storage and release of biogenic amines, such as 5-HT, blood platelets appear to share many characteristics with nerve endings (Pletscher, Da Prada & others, 1971). Therefore many investigators have attempted to correlate changes in uptake and release processes of 5-HT in human blood platelets with disorders of the central nervous system, such as infantile autism (Bouillin, Coleman & others, 1971), depressive illness (Coppen, 1968) or migraine (Deshmukh & Harper, 1973).

Generally, studies of 5-HT release from blood platelets *in vitro* have been carried out in incubation experiments. However, the principal drawback of such methods is that both release and (re)uptake occur simultaneously. Therefore an uptake inhibition may be misinterpreted as a releasing effect and vice versa. In this communication we present a simple superfusion method for blood platelets which circumvents the problems mentioned above.

Blood (20 ml) from rats of an inbred Wistar-strain (150–200 g) was collected in centrifuge tubes, which contained 2 ml of an anticoagulant (1% EDTA in 0·9% NaCl). Erythrocytes and white cells were separated by centrifugation at 200 *g* for 15 min. The platelet-rich supernatant was removed and diluted with an equal volume of 0·9% NaCl. Then the platelets were spun down at 800 *g* for 30 min, the clear supernatant was decanted and the pellet carefully resuspended in 4 ml of Krebs-Ringer-bicarbonate (KRB) medium. The KRB-medium had the following composition (mM): NaCl (118); KCl (4·85); $CaCl_2$ (2·5); $MgSO_4$ (1·15); KH_2PO_4 (1·15); $NaHCO_3$ (25); glucose (11·1); pH 7·2–7·4. After 10 min preincubation at 37° under an atmosphere of 5% CO_2 in oxygen in a Dubnoff metabolic incubator, 5 μCi ^{3}H-5-HT was added (final concentration 1–3 $\times$ 10^{-7}M) and the incubation was continued for 15 min. Then the platelets were collected by centrifugation at 800 *g* for 30 min and the pellet was resuspended in 250 μl KRB medium. 50 μl of this suspension was applied to each of the four chambers of the superfusion system.

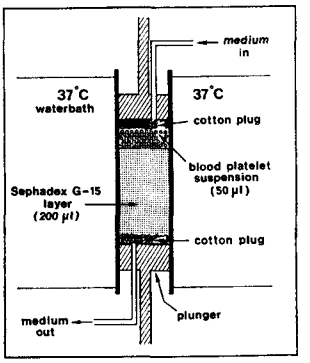

FIG. 1. Schematic drawing of one of the four superfusion chambers of the system. Basically, it consists of a syringe with two movable plungers. Well-oxygenated medium is pumped through the chambers at a rate of 0·25 ml min^{-1} and collected in 2 min fractions in vials, which are used for liquid scintillation counting of radioactivity.

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The superfusion chambers (Fig. 1) consist of syringes surrounded by a thermostated waterbath. The particle suspension was layered on top of a small column (200 μl) of Sephadex G-15, which was swollen in KRB medium the day before, and superfused with oxygenated medium at a rate of 0·25 ml min^{-1}, maintained by a peristaltic pump (Gilson, minipuls, MP-4). The effective volume above the Sephadex layer was adjusted by the movable plunger to about 50 μl. Fractions were collected every 2 min and the radioactivity was determined by liquid scintillation counting. A fairly constant rate of efflux of radioactivity was generally obtained within 40 min. At 50 min after starting the superfusion, the effect of drugs on ^{3}H-5-HT efflux was examined by superfusion for 10 min with KRB medium containing these drugs. Thereafter superfusion was continued for another 10 min with normal medium. The radioactivity in the effluent was analysed chromatographically on small Dowex-X_4 columns according to Kehr (1974).

Both tyramine (5×10^{-5}M) and reserpine (10^{-6}M) induced a strong increase in the efflux of radioactivity from blood platelets, labelled previously with ^{3}H-5-HT, although the time course of these drug effects was different (Fig. 2). After the addition of tyramine to the superfusion medium, an immediate sharp increase in the efflux of radioactive material was observed. On the other hand, under these circumstances reserpine induced a gradual increase. This reflects the different mechanisms by which these drugs interfere with the 5-HT storage pool within the platelets. Tyramine probably interferes with 5-HT-ATP-divalent cation aggregates present within the storage granules (Pletscher & others, 1971) resulting in a displacement of bound 5-HT. Reserpine is thought to inhibit the uptake of 5-HT into the granular stores, thus disturbing the balance of 5-HT fluxes between the granular and extra-granular amine pools. This results in an increased net efflux of 5-HT from the granular pool, which is observed as a gradual release of radioactivity from the platelets (Fig. 2). Analysis of the effluent radioactivity revealed that more than 65% of both basal and tyramine- or reserpine-induced efflux consisted of unmetabolized ^{3}H-5-HT. This figure differs considerably from that obtained in studies on radiolabelled 5-HT release from synaptosomes (Mulder, van den Berg & Stoof, 1975) where the percentage of unmetabolized ^{3}H-5-HT in the basal efflux was found to be approximately 30%. This indicates that the deaminating system in blood platelets is less active than in nerve endings.

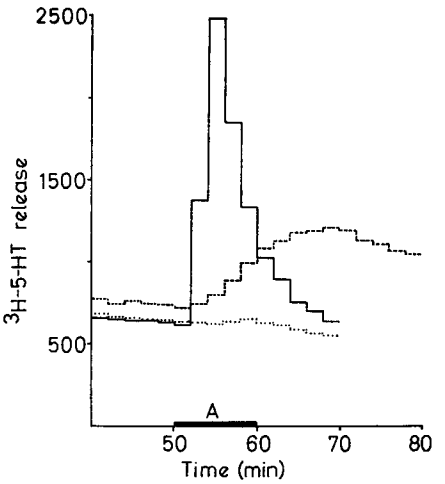

FIG. 2. Effects of tyramine (5×10^{-5}M) —, reserpine (10^{-6}M) - - - - and imipramine (10^{-6}M) — · — · — · on the efflux of radioactivity from blood-platelets preloaded with ^{3}H-5-HT (d min^{-1} per 2 min). A, drug present. — Tyramine, — — — Reserpine, · · · Imipramine.

During superfusion the blood platelets are present in a very small volume of medium (50 μl), which is continuously renewed at a comparatively high rate, thereby reducing the possibility of reuptake of released 5-HT to a minimum. In accordance with this, imipramine in a concentration (10^{-6}M) which is known to cause an effective inhibition of 5-HT uptake by platelets (Tuomisto, 1974) did not change the efflux of radiolabelled 5-HT during superfusion (Fig. 2).

The simple and reproducibly working superfusion system presented in this communication may be a valuable tool in studies on release of 5-HT from blood platelets without interference of reuptake processes.

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