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Subcellular distribution of some phenothiazines in blood platelets of rabbit

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Platelets incubated in plasma containing 5×10^{-5} and 3×10^{-4} M chlorpromazine, desmonomethylchlorpromazine or chlorpromazine sulphoxide were homogenized and fractioned by differential centrifugation. After fractionation most of the accumulated chlorpromazine and desmonomethylchlorpromazine were found in the particulate fractions, while most of the chlorpromazine sulphoxide was in the supernatant. The phenothiazines studied had a certain affinity for that fraction containing most of the platelet 5-hydroxytryptamine (5-HT). These phenothiazines released half of the platelet 5-HT without altering its intracellular distribution. The distribution of 5-HT was affected only by concentrations of chlorpromazine and desmonomethylchlorpromazine which released nearly all of the platelet 5-HT.

CHLORPROMAZINE and some other phenothiazines are known to cause 5-hydroxytryptamine (5-HT) liberation from blood platelets *in vitro* (Bartholini, Pletscher & Gey, 1961; Paasonen, 1964, 1965; Ahtee & Paasonen, 1965). Alterations in the chemical structure change the ability of these compounds to release 5-HT (Ahtee, 1966). The platelets accumulate many times more phenothiazines than are present in plasma, and there are certain relations between the ability of various compounds to liberate 5-HT from platelets and the uptake of phenothiazines by platelets (Ahtee & Paasonen, 1966). Most of the platelet 5-HT is bound in a certain granule fraction which can be separated by differential centrifugation (Solatunturi & Paasonen, 1966). Using similar fractionation the present experiments were undertaken to study how some of the phenothiazine derivatives are distributed in platelets.

Experimental

Male albino rabbits weighing 2·8–3·4 kg were bled under ether anaesthesia from the carotid artery through a polyethylene cannula. The platelet-rich plasma was obtained as described by Paasonen (1964) and 5 or 10 ml of it was incubated in air with gentle shaking at 37°. One ml of platelet-rich plasma contained $9{\cdot}5 \times 10^8 \pm$ s.d. $2{\cdot}6 \times 10^8$ platelets ($n = 19$). The experiments were made in polyethylene or polypropylene vessels, and polypropylene pipettes were used. Into each ml sample was added 0·1 ml of the drug solution or saline.

The drugs used were: chlorpromazine hydrochloride (May & Baker Ltd., Dagenham), desmonomethylchlorpromazine maleate and chlorpromazine sulphoxide (Rhône-Poulenc, Paris).

After incubation for 1 hr the platelets were separated by centrifugation at 4,000 *g* for 20 min at a temperature below 5°, and homogenized in 4 ml of 0·32M sucrose by ultrasound (Branson Sonifer S-75 with a $\frac{1}{8}$ inch micro tip, setting No. 3, 1 min). After homogenization 5 ml of 0·32M sucrose was added. The diluted homogenates were fractionated by differential

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centrifugation (Buckingham & Maynert, 1964; Solatunturi & Paasonen, 1966). From the particulate fractions of 2,500, 18,500 and 100,000 *g* sediments, and from the supernatant obtained, the phenothiazines were estimated spectrophotometrically (Salzman & Brodie, 1956) and 5-HT spectrophotofluorometrically (Bogdanski, Pletscher & others, 1956).

Results and discussion

DISTRIBUTION OF PHENOTHIAZINES

After incubation of the platelet-rich plasma with 5×10^{-5}M concentrations of phenothiazines, the platelets accumulated per ml of platelet-rich plasma $2{\cdot}0 \pm 0{\cdot}8$ μg (mean $\pm$ s.d., $n = 4$) of chlorpromazine, $3{\cdot}7 \pm 2{\cdot}6$ μg of desmonomethylchlorpromazine and $4{\cdot}1 \pm 0{\cdot}8$ μg of chlorpromazine sulphoxide. The corresponding accumulation in the platelets incubated with 3×10^{-4}M concentrations were $11{\cdot}4 \pm 3{\cdot}4$ μg, $13{\cdot}3 \pm 6{\cdot}2$ μg and $15{\cdot}5 \pm 4{\cdot}9$ μg, respectively. Fig. 1 represents the percentage distribution of these compounds in particulate fractions and

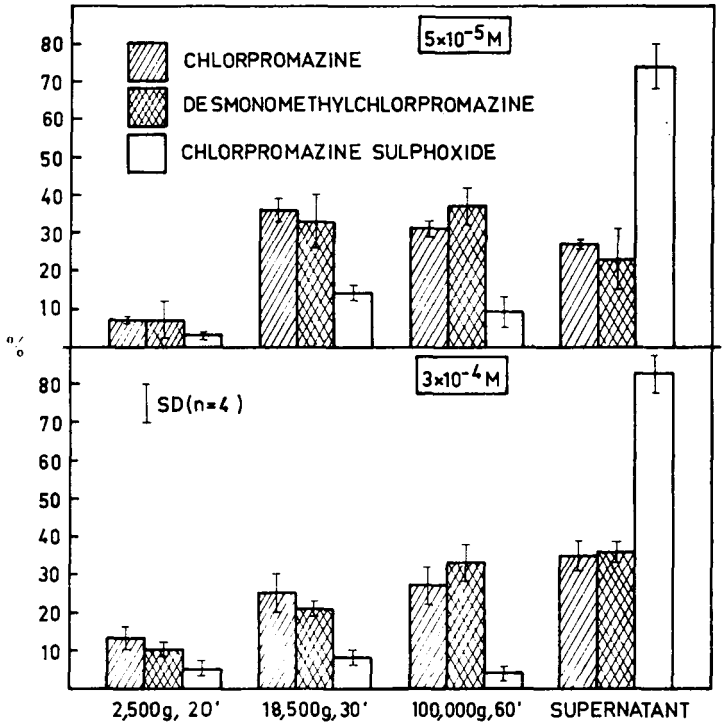

FIG. 1. Percentage distribution of phenothiazines in subcellular fractions of rabbit platelets incubated for 1 hr with 5×10^{-5} and 3×10^{-4}M concentrations of these compounds. Fractions were obtained by differential centrifugation of platelet homogenate. The three particulate fractions are indicated by the *g* values and the centrifugation times used to sediment them.

the supernatant after incubation of platelet-rich plasma with the two concentrations of phenothiazines mentioned above. After incubation with the higher concentrations the percentage of phenothiazines had increased more in the supernatant and in the 2,500 *g* sediment than in the other two fractions.

The distribution of chlorpromazine and its desmonomethyl derivative was similar in the supernatant and the particulate fractions, but they were present in much larger amounts in the particulate fractions. On the other hand, most of chlorpromazine sulphoxide was present in the supernatant fluid. These findings are related in the same way as is the behaviour of the three compounds on the central nervous system, chlorpromazine sulphoxide possessing much weaker central nervous system effects than chlorpromazine and desmonomethylchlorpromazine.

Among the particulate fractions, most of the phenothiazines were found in the 18,500 and 100,000 *g* sediments. The 100,000 *g* sediment contains microsomes and membranes but no 5-HT. On the other hand, the 18,500 *g* sediment contains two-thirds of the platelet 5-HT (Solatunturi & Paasonen, 1966). It is therefore interesting to note that this granule fraction also accumulated considerable portions of the phenothiazines. In this fraction 36% of the accumulated chlorpromazine, 33% of the desmonomethylchlorpromazine and 14% of the chlorpromazine sulphoxide were found after incubation with 5×10^{-5}M concentrations. After incubation with 3×10^{-4}M concentrations the corresponding percentages were about one-third lower.

To test the experimental procedure, the platelet-rich plasma was incubated without phenothiazine and the platelets from 5 ml of platelet-rich plasma were then homogenized in sucrose solution containing 60 μg of chlorpromazine. This is equal to the amount accumulated in the platelets from the 3×10^{-4}M incubation concentration. The distribution of chlorpromazine in subcellular fractions was similar to that presented above. Therefore the subcellular distribution of phenothiazines may be modified by the fractionation method used.

When the platelets incubated with 3×10^{-4}M chlorpromazine, desmonomethylchlorpromazine or chlorpromazine sulphoxide were washed twice with saline, their total phenothiazine contents decreased by 66, 51 and 90%, respectively. However, the percentages of these phenothiazines remaining in the platelets after washing were larger than initially in the 18,500 *g* sediment. In this fraction before washing there was 25% of the accumulated chlorpromazine, 21% of the desmonomethylchlorpromazine and 8% of the chlorpromazine sulphoxide. After two washings these relative phenothiazine amounts were increased to 30, 35 and 15%, respectively. In the other fractions, especially in the supernatant, the relative amounts of the phenothiazines correspondingly decreased. An exception was the 2,500 *g* sediment from chlorpromazine-treated platelets, in which the percentage of chlorpromazine also slightly increased, possibly because this fraction contains some poorly homogenized cells among the subcellular particles. The above experiments suggest that chlorpromazine and related phenothiazines have a certain affinity for the 18,500 *g* sediment.

EFFECT OF PHENOTHIAZINES ON THE DISTRIBUTION OF 5-HT

The effect of phenothiazines on the intracellular distribution of 5-HT in platelets was also studied. During 1 hr incubation of platelet-rich plasma, none of the phenothiazines released 5-HT from platelets in the concentration of 5×10^{-5}M. In 3×10^{-4}M concentration chlorpromazine released 37%, desmonomethylchlorpromazine 73% and chlorpromazine sulphoxide 24% of the platelet 5-HT. In a concentration of 10^{-3}M, chlorpromazine caused a release of 98%. This agrees with earlier data (Ahtee, 1966). In 5×10^{-5}M concentration none of the phenothiazines, and in 3×10^{-4}M concentration only chlorpromazine and chlorpromazine sulphoxide did not alter the percentage distribution of 5-HT in the above fractions. However, in concentrations which release nearly all of the platelet 5-HT, chlorpromazine and desmonomethylchlorpromazine caused changes also in the intracellular distribution of this amine. The 18,500 *g* sediment contained 60% of the platelet 5-HT in controls incubated without phenothiazines. Incubation for 1 hr with 10^{-3}M chlorpromazine lowered the proportion of 5-HT in this fraction to 35% and incubation with 3×10^{-4}M desmonomethylchlorpromazine lowered it to 48% of the total platelet 5-HT found after these treatments. Correspondingly the relative amount of 5-HT in the supernatant increased.

The phenothiazines were able to release half of the platelet 5-HT without altering its intracellular distribution. Therefore, it is plausible that they exert their effect both on the cell membrane and on intracellular 5-HT-containing structures. The fractionation method used may have modified the subcellular distribution of phenothiazines. However, the potent 5-HT releasers, chlorpromazine and desmonomethylchlorpromazine, seem to possess a certain affinity for the 18,500 *g* sediment, which contains most of the platelet 5-HT.

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