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TEMPERATURE DEPENDENCE OF THE PASSIVE IN- AND OUTFLOW OF 5-HYDROXYTRYPTAMINE IN BLOOD PLATELETS

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Abstract—In isolated blood platelets of rabbits, the passive in- and outflow of 5-hydroxytryptamine (5HT)—as measured under various experimental conditions (i.e. in the absence of glucose, after reserpine treatment, with metabolic inhibitors)—is markedly decreased by lowering the incubation temperature. The 5HT liberation induced by sympathomimetic amines and reserpine shows a similar temperature sensitivity which is independent of the mechanism of action of the drugs on 5HT storage and may therefore be due to alterations in the passive outflow of liberated 5HT. From these experiments, it seems possible that changes of the passive 5HT in- and outflow also occur *in vivo*, especially in hypo- and hyperthermia.

MARKED temperature dependence of biological transport phenomena (e.g. uptake and storage of monoamines) frequently indicates the presence of active (energy-requiring) mechanisms. In a preliminary report it has, however, been demonstrated that the outflow of 5HT from isolated blood platelets of rabbits is highly dependent upon temperature under conditions in which energy-requiring transport mechanisms do not seem to be involved.[1]

This paper presents a more complete report on the temperature dependence of the passive in- and outflow of 5HT in blood platelets under various experimental conditions.

EXPERIMENTAL

Blood platelets of rabbits were isolated as previously described,[2] and the following experiments were carried out at pH 7·5:

1. Pre-incubation of platelets in glucose-free isotonic K-phosphate at 37°C for 1 hr, resuspension of the platelets in new buffer and incubation for various times at 37°C and for 120 min at various temperatures with and without presence of ouabaine.
2. Incubation of platelets at various temperatures in modified Tyrode solution* and plasma supplemented with KCN, *p*-chloro-N-methyl-β-phenethylamine (Ro 4-6861), tyramine or reserpine
3. Pre-incubation of platelets at 10° in Tyrode solution supplemented with CH_3HgI for ½ hr; re-incubation of the centrifuged washed platelets in Tyrode solution or plasma at various temperatures.

* NaCl	7·60 g/l	$NaHCO_3$	2·10 g/l
KCl	0·42 g/l	Glucose	2·00 g/l
Versene	0·80 g/l	Sucrose	4·50 g/l
$NaH_2PO_4.2H_2O$	0·14 g/l.		

4. Pre-incubation of platelets from reserpinized rabbits (treated with 5 mg reserpine/kg i.v. 16 hr previously) in Tyrode solution at 37° with 500 μg/ml 5HT for $\frac{1}{2}$ hr; resuspension of the centrifuged platelets in Tyrode solution and incubation at various temperatures.
5. Pre-incubation of platelets from reserpinized rabbits (treated as outlined under 4) for 1 hr in glucose-free K-phosphate. Re-incubation of the platelets with 500 μg/ml 5HT in glucose-free K-phosphate for $\frac{1}{4}$ hr at various temperatures. Before measuring the 5HT content of the platelets, they were washed twice with ice-cold K-phosphate.
6. Measurement of the inflow of ^{3}H-labelled* Ro 4-6861 into platelets suspended in Tyrode solution at various temperatures. After incubation for 120 min, the platelets of 2 ml suspension were separated from the medium by centrifugation, washed in ice-cold Tyrode, homogenized with 0·1 ml $HClO_4$ (30%) plus 1·4 ml HCl (0·1 N) and recentrifuged. The total radioactivity of the supernatant was measured in a liquid scintillation spectrometer.

If not otherwise stated, the suspensions contained twice the platelet amount of the original plasma. The 5HT of the platelets was measured by a spectrophotofluorimetric procedure.[3] The difference in the 5HT values of platelets (contained in 1 ml suspension) before and after incubation was considered as the amount of amine having left the platelets (outflow of 5HT).

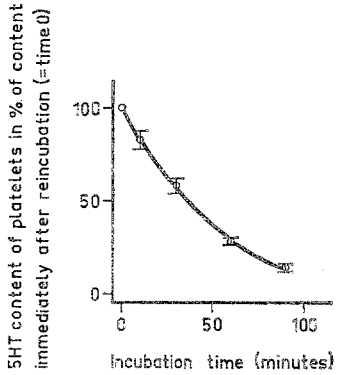

FIG. 1. Spontaneous liberation of 5-hydroxytryptamine (5HT) from isolated blood platelets of rabbits pre-incubated in glucose-free K-phosphate for 60 min at 37° and re-incubated in new buffer at 37°. Each point indicates an average of 3 experiments ± S.E. Absolute 5HT value of platelets immediately after re-incubation (controls): 12·2 ± 1·1 μg/ml.

RESULTS

Platelets pre-incubated in glucose-free K-phosphate rapidly lose the 5HT if they are re-incubated in new buffer. After 90 min, their 5HT content is only 14 ± 1% of that present at the beginning of the re-incubation period (Fig. 1). This 5HT outflow is not modified by ouabaine in concentrations of 10–250 μg/ml. A similar marked 5HT depletion can be seen if the platelets are incubated with KCN in Tyrode or plasma

* Prepared by Drs. H. Bruderer and J. Würsch, Chemical Research Department, F. Hoffmann-La Roche & Co. Ltd., Basle. ^{3}H-labelled in α-position of the amino group.

and if the platelets are preincubated with CH_3HgI and re-incubated in Tyrode or plasma. Furthermore, platelets of reserpinized rabbits loaded with exogenous 5HT (by previous incubation with 500 μg/ml 5HT) also lose a considerable part of the amine on re-incubation in plasma or Tyrode (Table 1).[1]

TABLE 1. EFFECT OF TEMPERATURE ON THE 5-HYDROXYTRYPTAMINE (5HT) OUTFLOW FROM PLATELETS OF RABBITS INCUBATED IN TYRODE AND PLASMA UNDER VARIOUS EXPERIMENTAL CONDITIONS

Compounds	Concentr. of compounds (μg/ml)	Medium	Incub. time (min)	5HT outflow		
				37°	20°	20°/37°
CH_3HgI*	60	Tyrode	90	81 ± 7	27 ± 7	0·32 ± 0·07
		Plasma	90	79 ± 4	23 ± 8	0·28 ± 0·08
KCN	400	Tyrode	90	80 ± 4	1 ± 1	0·02 ± 0·01
		Plasma	90	78 ± 7	10 ± 3	0·13 ± 0·04
Ro 4-6861	140	Tyrode	120	79 ± 2	13 ± 4	0·16 ± 0·05
		Plasma	120	52 ± 5	4 ± 1	0·08 ± 0·02
Tyramine	200	Tyrode	120	66 ± 1	13 ± 5	0·19 ± 0·08
	300	Plasma	120	53 ± 10	3 ± 1	0·07 ± 0·04
Reserpine	5	Tyrode	120	35 ± 3	5 ± 2	0·14 ± 0·07
	5	Plasma	120	36 ± 4	0 ± 5	0·01 ± 0·08
Exogenous 5HT† in reserpinized platelets	500	Tyrode	120	76 ± 3	37 ± 8	0·47 ± 0·08
		Plasma	120	73 ± 4	29 ± 4	0·40 ± 0·07

The figures indicate the percentage of the platelet 5HT which left the platelets during the incubation period. Each figure represents an average of 3–8 experiments ± S.E.

* Pre-incubation with CH_3HgI in Tyrode at 10° for 30 min.

† Rabbits treated with 5 mg reserpine/kg i.p. before isolation of the platelets. Platelets pre-incubated with 500 μg/ml 5HT in Tyrode for 30 min at 37°, washed with Tyrode and re-incubated in Tyrode or plasma respectively. Absolute concentration of platelet 5HT before re-incubation: 18·4 ± 1·5 μg/ml (the suspension contained 3 times the amount of platelets as the original plasma).

The outflow of the 5HT from the platelets is very sensitive to temperature. Thus, in platelets suspended in glucose-free K-phosphate, incubation at 33° decreases the outflow to about 50% (as compared to controls incubated at 37°), and at 20° it is practically abolished. Elevation of the temperature to 40° increases the outflow to 119%. The 5HT outflow from platelets pre-incubated with CH_3HgI and re-incubated in plasma shows a similar temperature dependence (Fig. 2). Furthermore, in all the experimental conditions as mentioned above) the 5HT outflow at 20° is markedly less than that at 37°. No principal difference exists whether the platelets are incubated in plasma or in Tyrode (Table 1).

The 5HT outflow from platelets induced by tyramine, Ro 4-6861 and reserpine is progressively diminished with decreasing temperature (Fig. 3). The percentage diminution is similar for all the three drugs. Thus, in Tyrode solution at 30° and 20°, only about 60% and 10% respectively of the 5HT leave the platelets as compared to the value at 37° (100%). In plasma the 5HT amount which leaves the platelets is decreased to less than 5% at an incubation temperature of 20° (Table 1).

Decrease of the temperature diminishes the inflow of Ro 4-6861 into the platelets to a relatively small degree (penetration of 83% at 20° in comparison to that at 37°) (Fig. 4).

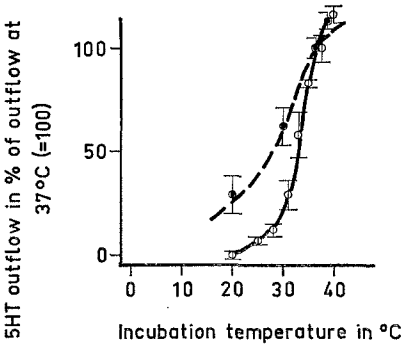

FIG. 2. Effect of temperature on the passive 5-hydroxytryptamine (5HT) outflow from isolated blood platelets of rabbits under different experimental conditions. ○——○ platelets pre-incubated in glucose-free K-phosphate for 60 min at 37° and re-incubated in new buffer for 60 min; ● - - - ● platelets pre-incubated with 20 μg/ml CH_4HgI in Tyrode, washed and re-incubated in plasma for 90 min. Each point indicates an average of 4–6 experiments ± S.E.

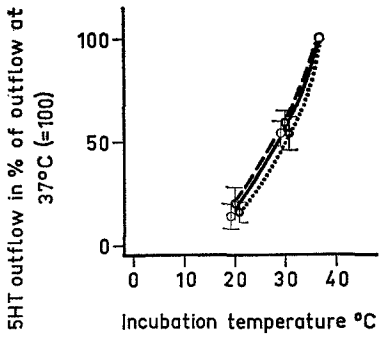

FIG. 3. Effect of temperature on the drug-induced 5-hydroxytryptamine (5HT) liberated from platelets of rabbits incubated in Tyrode at 37° for 120 min. ○——○ reserpine 5 μg/ml; ⊙ - - - - - ⊙ tyramine 200 μg/ml; **O**·····**O** Ro 4-6861 140 μg/ml. Each point represents an average of 3–7 experiments ± S.E. Absolute 5HT content of isolated platelets incubated for 120 min without drugs: 14·0 ± 0·8 μg/ml (the suspensions contained an equal amount of platelets per ml as the original plasma).

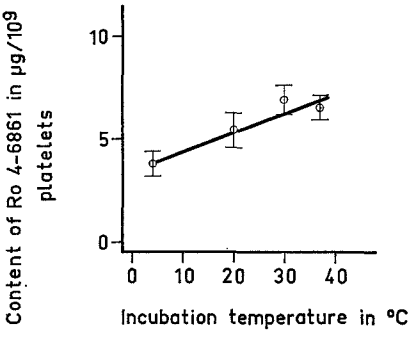

FIG. 4. Penetration of ^3H-labelled Ro 4-6861 into isolated blood platelets of rabbits incubated in Tyrode at various temperatures for 120 min. Concentration of Ro 4-6861 in the incubation medium: 86·5 μg/ml.

In glucose-free K-phosphate the inflow of 5HT into reserpinized platelets pre-incubated without glucose is markedly inhibited by decreasing the temperature. A reduction of the temperature to 32° causes an approximately 50% diminution of the 5HT penetration, whereas an increase to 40° enhances the inflow of the amine to 126% (Fig. 5).

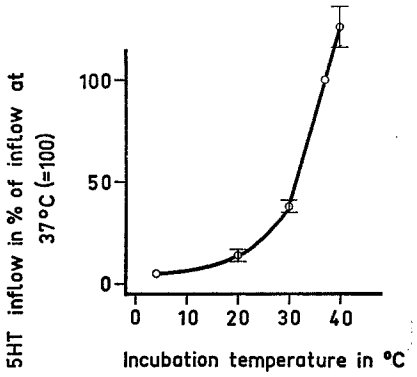

FIG. 5. Effect of temperature on the inflow of 5-hydroxytryptamine (5HT) into blood platelets from reserpinized rabbits (5 mg/kg reserpine i.p. 16 hr prior to the isolation of the platelets). The platelets were pre-incubated for 60 min in glucose-free K8phosphate and re-incubated for 15 min at various temperatures in new buffer containing 500 μg/ml 5HT. Prior to the 5HT determination, the platelets were washed twice with glucose-free K-phosphate. Each point indicates an average of 3 experiments ± S.E. Absolute 5HT content of platelets (a) before incubation with exogenous 5HT: 0·09 ± 0·01 μg/ml, (b) 15 min after incubation with 5HT at 37°: 8·95 ± 0·92 μg/ml.

DISCUSSION

Under physiological conditions—i.e. in plasma or glucose-containing buffers (e.g. Tyrode)—isolated blood platelets of rabbits lose at the most small amounts (1–5%) of their endogenous 5HT.[4, 5] This is probably due to storage of the amine in the platelets by an active process which maintains a concentration gradient between the platelets and the incubation medium.

The storage of 5HT is no longer possible in platelets incubated in glucose-free K-phosphate, with metabolic inhibitors or in reserpinized platelets having been loaded with exogenous 5HT. It may be assumed that under these conditions active processes which are responsible for the maintenance of high intracellular 5HT levels are abolished, e.g. by lack of energetic substrates (glucose), interference with the glucose metabolism (metabolic inhibitors) or by impairment of granular storage of 5HT (reserpine). Therefore, passive processes (e.g. diffusion of 5HT through membranes, absorption to macromolecules) seem to become the limiting factors for the 5HT outflow from the platelets. The missing effect of ouabaine also indicates a passive mechanism by which 5HT leaves the platelets incubated in glucose-free K-phosphate.

The experiments in which platelets were incubated with exogenous 5HT show that not only the outflow but also the inflow of 5HT is highly sensitive to changes of temperature (Fig. 5). It is unlikely that under the conditions of these experiments an active 5HT uptake occurs, since the platelets originate from reserpinized animals and since the incubation medium does not contain energy-supplying substrates such as glucose.

The temperature-dependence of the passive 5HT in- and outflow of platelets cannot be due to a mere alteration of the thermic molecular movements, because this mechanism would not explain the magnitude of the temperature effect in the above experiments. An artifact due to the synthetic media (e.g. K-phosphate, Tyrode buffer) is unlikely, because similar temperature effects can also be demonstrated with plasma. Since the in- as well as the outflow of 5HT is decreased by lowering of the temperature, it may be assumed that alterations in the physico-chemical properties of the platelet membranes are involved. It has, for instance, been shown that films of fatty acids and other lipids increase their surface area abruptly with temperature rising within a relatively narrow range.[6] Since membranes and other biological structures contain lipid constituents, similar changes might occur in the living cell. The quantitative differences in the temperature-induced changes of the 5HT outflow as seen under various experimental conditions (Table 1) might be due to a direct influence of drugs and metabolic inhibitors (e.g. reserpine, CH_3HgI) on membrane structures.

The present experiments also show that the temperature dependence of the 5HT outflow is independent of the type of drug-induced 5HT liberation. Thus, the sympathomimetic amines tyramine and Ro 4-6861 on the one hand and reserpine on the other hand probably liberate 5HT by different mechanisms, i.e. by displacement[7] and by interference with active storage[8] respectively. Despite this difference, the temperature dependence of the 5HT liberation is closely similar with the two types of drugs indicating that the passive outflow of the liberated 5HT might be altered. A major temperature dependence of the penetration of the drugs into the platelets is not likely, since measurements with ^{3}H-labelled Ro 4-6861 show only a relatively small diminution of its inflow with decreasing temperature (Fig. 4).

The high temperature dependence of passive mechanisms is not only seen with 5HT. Thus, in previous experiments the spontaneous liberation of norepinephrine from isolated granules of sympathetic nerves has been shown to be markedly diminished by lowering of the temperature.[9] This norepinphrine outflow is probably passive since under the conditions of the experiment (isolated granules in glucose-free K-phosphate) active storage and transport are unlikely to occur.

In conclusion, the high temperature sensitivity of the passive in- and outflow of monoamines *in vitro*, especially within the range of 30–40°, suggests the possibility of similar changes *in vivo*, e.g. in hypothermia or hyperpyrexia.

REFERENCES

1. M. DA PRADA, A. PLETSCHER and G. BARTHOLINI, *Life Sci.* 4, 1773 (1965).
2. G. BARTHOLINI and A. PLETSCHER, *Experientia* **20**, 376 (1964).
3. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **117**, 82 (1957).
4. G. BARTHOLINI and A. PLETSCHER, *Experientia* **20**, 376 (1964).
5. M. K. PAASONEN and A. PLETSCHER, *Experientia* **15**, 477 (1959).
6. N. K. ADAM, in *The Physics and Chemistry of Surfaces*, p. 60. Oxford University Press, London, (1941).
7. H. J. SCHÜMANN and A. PHILIPPU, *Nature, Lond.* **193**, 890 (1962).
8. P. A. SHORE, *Pharmac. Rev.* **14**, 531 (1962).
9. U. S. v. EULER and F. LISHAJKO, *Acta physiol. scand.* **57**, 468 (1963).