Increase of cyclic GMP in blood platelets by biogenic amines – a receptor-mediated effect?

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Various biogenic amines including 5-hydroxytryptamine (5-HT) and catecholamines raise the content of cyclic guanosine-1'5'-monophosphate (cGMP) in blood platelets (Agarwal & Steiner 1976; Jakobs et al 1974). These amines also cause functional changes of the platelets, e.g. aggregation and a shape change reaction, which are thought to be due to stimulation of receptors at the plasma membrane (Laubscher & Pletscher 1979; Drummond 1976; Boullin & Grimes 1976). The results of the present experiments indicate that the rise in cGMP induced in human platelets by biogenic monoamines is probably not mediated by interaction with specific receptors.

Blood platelets were obtained from healthy donors by puncturing a cubital vein. The whole blood was collected in a plastic bottle and mixed with 1/10 volume 3·8% Na-citrate . $2H_2O$. The platelets were isolated by means of dextran gradients and diluted with Tris-buffer to give a final concentration of 10^5 μl^{-1}, as described earlier (Graf et al 1979). Theophylline was added to the platelet suspension 1, 2 ml) to give a final concentration of 10^{-3} M. The platelets were preincubated for 60 min at 37 °C, then the amines and drugs, either alone or together with potential inhibitors, were added. The reaction was stopped after 3 min, if not indicated otherwise, by removing 1 ml of the platelet suspension into 1 ml of 3% perchloric acid. The cGMP and cyclic adenosine-1'5'-monophosphate (cAMP) were extracted according to the assay protocol from Collaborative Research Inc., Waltham, Ma., USA and determined by radioimmunoassay using the kits from Becton Dickinson Immunodiagnostics, Orangeburg, N.Y., USA. The chemicals used were obtained from commercial sources except for (+)-LSD and psilocin, which were generous gifts from Sandoz Inc. Basel. No aggregation of the platelets occurred during the experiments.

Various biogenic amines tested, i.e. 5-HT, dopamine (DA), noradrenaline (NA) and adrenaline (A) and the 5-HT agonist psilocin, caused a marked increase in the cGMP content of platelets, the most effective compounds being 5-HT and psilocin. As has been shown before (Agarwal & Steiner 1976) 5-HT exerted its maximal action after about 30 s with the cGMP remaining considerably elevated for at least 4 min afterwards; increasing concentrations of 5-HT caused a progressive rise of cGMP (Fig. 1). The cAMP content of the platelet was not markedly changed either by 5-HT or by the catecholamines (Table 1).

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There is evidence that the above-mentioned amines and psilocin interact with specific amine receptors (5-HT, DA, NA) on the platelet membrane (Laubscher & Pletscher 1979; Drummond 1976; Boullin & Grimes 1976). However, the present findings indicate that these compounds probably do not alter the cGMP level in platelets by stimulating the specific amine receptors. Firstly, other amines, e.g. tryptamine, *NN'*-dimethyltryptamine and (+)-lysergic acid diethylamide (LSD), which have been shown to stimulate 5-HT receptors at the platelet membrane (Laubscher & Pletscher 1979), did not cause a significant ($P < 0.01$) increase in cGMP. This indicates that an OH-group in the 4 or 5 position of the indole ring is necessary for the elevation of the cGMP but not for the stimulation of the 5-HT receptor. Also, neither metergoline in concentrations as high as 10^{-5} M nor methysergide (Agarwal & Steiner 1976) markedly reduced the 5-HT-induced rise of cGMP, whereas both were potent in blocking the 5-HT receptor (order of potency 10^{-8} M in inhibiting the 5-HT-induced shape change) (Laubscher & Pletscher 1979). Secondly, although the DA receptor antagonist

Table 1. Effect of various substances on cyclic nucleotides in human platelets. The contents of cyclic nucleotides are indicated in percent of controls (= 100%). Each value is an average with s.e.m. of 3–6 experiments. Absolute controls in pmol/10^9 platelets: cAMP 17·89 ± 0·98 (n = 10), cGMP 3·84 ± 0·40 (n = 23). Unless otherwise stated the concentration of the drugs was 10^{-5} M.

Substance	cAMP %	cGMP %
Agonists		
5-HT (10^{-6} M)	104 ± 5	387 ± 37
5-HT (10^{-5} M)	106 ± 3	1477 ± 450
Psilocin		1381 ± 420
Tryptamine		113 ± 12
NN'-Dimethyltrypt.		114 ± 11
(+)-LSD		66 ± 3
Dopamine	100 ± 7	634 ± 93
(—)-Noradrenaline	90 ± 9	434 ± 41
(+)-Noradrenaline		454 ± 95
(—)-Adrenaline	99 ± 1	449 ± 7
Antagonists		
Imipramine		347 ± 27
Amitryptiline		84 ± 9
Chlorpromazine		629 ± 137
Spiroperidol		48 ± 3
Methysergide		547 ± 147
Metergoline		105 ± 15
Phenoxybenzamine		50 ± 7
Phentolamine		177*

* 1 experiment

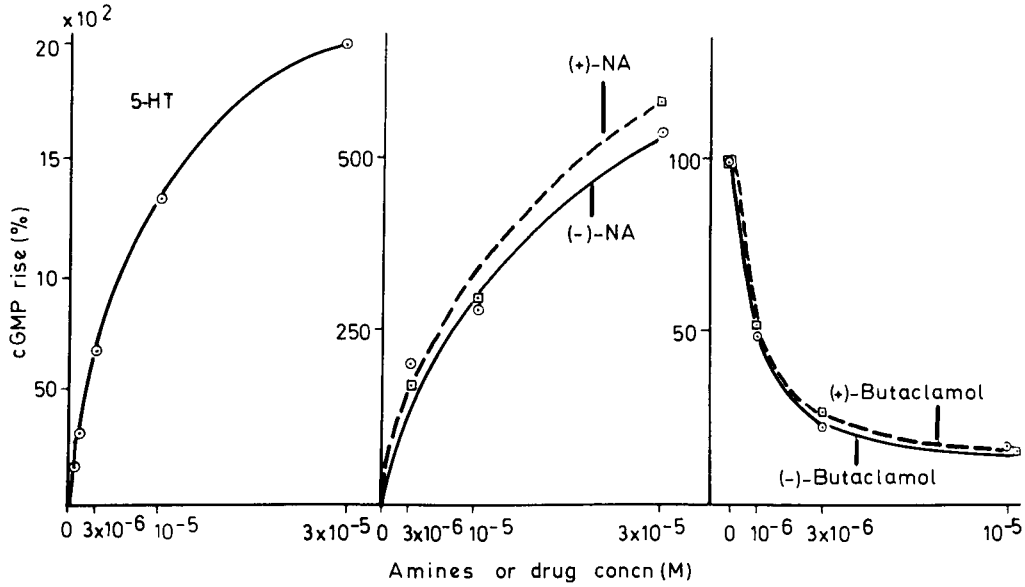

FIG. 1. Effect of various concentrations of 5-hydroxytryptamine (5-HT), (+)- and (—)-noradrenaline (NA) on cGMP and action of (+)- and (—)-butaclamol on cGMP stimulated by 10^{-5} M dopamine (DA); human platelets. In the 5-HT and NA experiments the values are indicated in percent of those in control platelets (= 100%). In the butaclamol experiment the value obtained on stimulation with DA alone was taken as 100%. Single experiments, each value is the average of duplicate measurements.

butaclamol antagonized the DA-induced increase of cGMP this action was not stereospecific, (+)- and(—)-butaclamol being equally effective. Thirdly, the rise of cGMP caused by NA did not show stereoselectivity, the actions of (+)- and (—)-NA being similar (Fig. 1). Finally, various compounds which block amine receptors influenced cGMP in different ways. Thus, some antagonists of 5-HT, DA and NA (methysergide, chlorpromazine and phentolamine) caused an increase of cGMP, whereas others (metergoline, spiroperidol and phenoxybenzamine) had either no influence or exerted a lowering effect. Also, imipramine and amitryptiline, which, besides their inhibitory effect on 5-HT uptake also block 5-HT receptors on platelets (Laubscher & Pletscher 1979) either increased or did not change the cGMP (Table 1).

The mechanism by which agonists and antagonists of amine receptors influence the cGMP in platelets is not clear. These substances could change the intracellular calcium, which might influence the activity of guanylate cyclase (reviewed by Greengard 1979). Another possibility is that the receptor agonists and antagonists act on guanylate cyclase directly. This view is supported by previous findings, which showed a non-stereoselective activation by catecholamines of soluble (cytosolic) guanylate cyclase from the renal cortex of rabbits (Liang & Sacktor 1978).

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