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Effect of 5-hydroxytryptamine on blood glucose and cyclic AMP in the rat

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The effects of 5-hydroxytryptamine (5-HT) on plasma cyclic AMP (cAMP) and glucose concentrations were studied in rats in vivo. 5-HT injected i.p. increased plasma cAMP and glucose. Injections of propranolol, hexamethonium, and cyproheptadine inhibited the 5-HT-induced increase in glucose but not in cAMP. Atropine did not inhibit the action of 5-HT. These effects of 5-HT were not seen in adrenomedullectomized rats, and 5-HT did not elevate the concentration of plasma cAMP in anti-glucagon antiserum-injected rats. These results confirm the previously reported finding that 5-HT-induced increase in blood glucose is mediated via adrenaline released from adrenal medulla by 5-HT and suggest that the increase in plasma cAMP, induced by 5-HT, is due to glucagon released by an unknown factor, or factors other than adrenaline released from the adrenal medulla by 5-HT.

Kobayashi et al (1960) reported that 5-hydroxytryptamine (5-HT) produced significant hyperglycaemia in intact rats and that this effect was due to secondary liberation of adrenaline from the adrenal medulla in vivo. In rat adipose tissues, 5-HT stimulated glucose uptake (Vaughan 1961) and release of free fatty acids (Itaya & Ui 1964; Yoshimura et al 1969; Itaya 1978a,b) production of which is mediated by cyclic AMP (cAMP). As little has been published on the effect of 5-HT on the blood concentration of cAMP, we have studied whether 5-HT increases cAMP in rat blood and how the hyperglycaemia observed after 5-HT administration correlates with the plasma cAMP.

MATERIALS AND METHODS

Male rats, Wistar-derived, 250 to 350 g, maintained on a standard pellet diet were allowed free access to food and water. Blood sugar determinations were made by the method of Bergmeyer & Bernt (1963) on 0·02 ml of whole blood from the tail vein. cAMP in plasma was determined by radioimmunoassay (Honma et al 1977). Blood glucose was expressed as mg % whole blood, and cAMP as pmol ml^{-1} of plasma.

All drugs, except anti-glucagon antiserum, were injected intraperitoneally at the doses indicated in each Figure. The drugs were: 5-HT (as creatinine sulphate), atropine (Sigma Chemical Co.), adrenaline (Merck, Sharp and Dohme), hexamethonium (Nakarai Chemicals, Ltd., Tokyo), propranolol (a gift from Ohtsuka Pharmaceutical Co., Tokushima), cyproheptadine (Merck-Banyu Co., Tokyo), glucagon (a gift from Eli Lilly and Co.) and anti-glucagon rabbit antiserum (a gift from Ohtsuka Pharmaceutical Co.). Other reagents were of analytical grade from commercial sources.

In some experiments, rats bilaterally adrenomedullectomized (under pentobarbitone anaesthesia) were used 4 days after the operation. Anti-glucagon antiserum or normal serum was infused i.v. at 0·7 ml of serum h^{-1} into the rats under pentobarbitone.

RESULTS

Increases in plasma cAMP and blood glucose after an intraperitoneal injection of 5-HT. 5-HT injected i.p. at 4 mg kg^{-1}, as expected increased the concentration of plasma cAMP (Fig. 1). At the same time, blood glucose was increased but the rise occurred more slowly than that of cAMP.

Effect of a β-adrenoceptor blocking agent (propranolol) on the effect of 5-HT and adrenaline. The results above suggest that the adrenaline released from the adrenal medulla by 5-HT (Kobayashi et al 1960) stimulates cAMP and glucose formation. If this is so, the effects of 5-HT on blood cAMP and glucose concentrations would be diminished by pretreatment of the rats with a β-adrenoceptor blocking agent because the stimulatory-effects of adrenaline on plasma cAMP and glucose would be blocked. However, the effect of 5-HT on cAMP was present in the rats preinjected with propranolol (5 mg kg^{-1}), whereas blood glucose was not increased by 5-HT.

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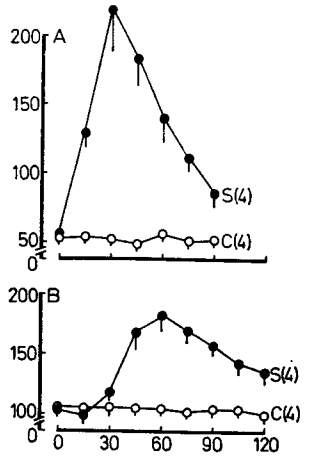

FIG. 1. Effect of 5-HT on plasma cAMP and glucose concns. 5-HT, 4 mg kg^{-1}, was injected i.p. at 0 min. C, control; S, 5-HT. Number of animals used are shown in parentheses. Vertical lines represent the s.e.m. Ordinate: in A, cAMP (pmol ml^{-1} plasma); in B, glucose (mg %). Abscissa: time (min).

Differential effect of hexamethonium on cAMP elevated by 5-HT from that on glucose increase by 5-HT. The possible involvement of endogeneous adrenaline released from the adrenal medulla in the 5-HT-induced increase in cAMP and glucose was studied with hexamethonium which inhibits the release of adrenaline from the adrenal medulla. If the effect of 5-HT on blood cAMP and glucose is mediated by adrenaline released from adrenal medulla, hexamethonium-injected rats should be unable to respond to 5-HT. However hexamethonium did not diminish the effect of 5-HT on cAMP concentration but did inhibit 5-HT's effect on blood glucose (Fig. 2). This suggests that the 5-HT-induced increase in plasma cAMP is not mediated by adrenergic nerves, nor is it mediated by cholinergic nerves because atropine did not inhibit the action of 5-HT on cAMP or glucose.

The effect of cyproheptadine on 5-HT- and adrenaline-induced increase in blood concentrations of cAMP and glucose. Propranolol and hexamethonium (Fig. 2) inhibited the effect of 5-HT only on blood glucose. However, as cyproheptadine, a 5-HT antagonist, may inhibit both the actions of 5-HT, it was injected at 4 mg kg^{-1} 20 min before 5-HT, but it was without effect on plasma cAMP (Fig. 3). although it diminished the action of 5-HT in increasing blood glucose. Cyproheptadine had no effect on adrenaline's (100 μg kg^{-1}) action on glucose or cAMP in blood.

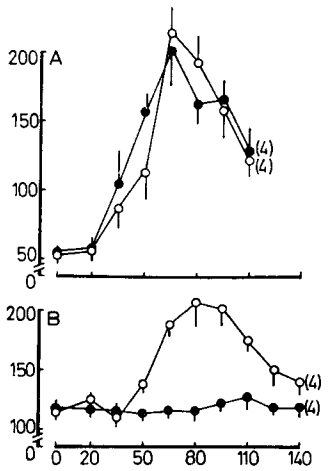

FIG. 2. Difference of the effect of hexamethonium on 5-HT-induced increase in plasma cAMP and on that in blood glucose. ○—○, saline, 1ml kg^{-1}, was injected i.p. at 0 min, and 5-HT, 4 mg kg^{-1}, at 20 min. ●—●, hexamethonium, 10 mg kg^{-1}, was injected i.p. at 0 min, and 5-HT, 4 mg kg^{-1}, at 20 min. Hexamethonium only had no effects on cAMP and glucose concentrations. Number of animals, vertical lines, ordinate and abscissa as for Fig. 1.

Failure of 5-HT to increase plasma cAMP in adrenomedullectomized rats. 5-HT failed to increase plasma cAMP in adrenomedullectomized rats (Fig. 4). This means that the 5-HT-induced increase in plasma cAMP is mediated in some way other than by

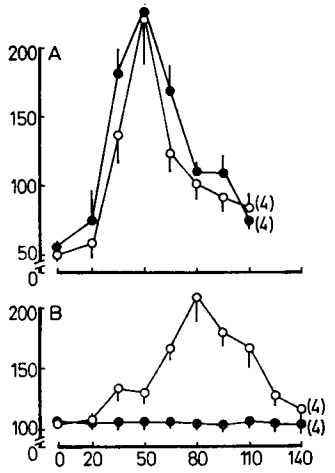

FIG. 3. Difference of the effect of cyproheptadine on 5-HT-induced increase in plasma cAMP and that in blood glucose. ○—○, saline, 1 ml kg^{-1}, was injected i.p. at 0 min, and 5-HT, 4 mg kg^{-1}, at 20 min. ●—●, cyproheptadine, 4 mg kg^{-1}, at 0 min, and 5-HT, 4 mg kg^{-1}, at 20 min. Cyproheptadine only had no effects on cAMP and glucose concentrations. Number of animals, vertical lines, ordinate and abscissa as for Fig. 1.

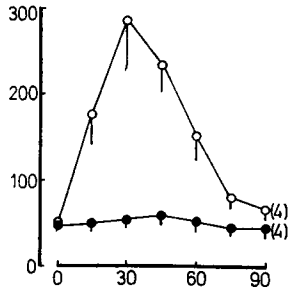

FIG. 4. Failure of 5-HT to increase plasma cAMP in adrenomedullectomized rats. Injections of 5-HT, 4 mg kg^{-1}, were performed i.p. at 0 min. ○—○, intact rats; ●—●, adrenomedullectomized rats. Number of animals, vertical lines, and abscissa as for Fig. 1. Ordinate: cAMP (pmol ml^{-1} plasma).

adrenaline released from the adrenal medulla by 5-HT, because propranolol failed to inhibit the increasing action of 5-HT in plasma cAMP.

Disappearance of 5-HT effect on plasma cAMP in rats infused with anti-glucagon antiserum. The infusion of anti-glucagon antiserum diminished the ability of 5-HT to increase plasma cAMP (Fig. 5). Control serum had no effect on 5-HT action.

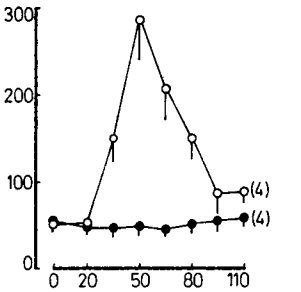

FIG. 5. Effect of infusion of anti-glucagon antiserum on 5-HT-induced increase in plasma cAMP. The normal rabbit serum or anti-glucagon rabbit antiserum was infused i.v. (0·7 ml h^{-1}) into intact rats during experiment. 5-HT, 4 mg kg^{-1}, was injected i.p. at 20 min. In this experiment, propranolol, 5 mg kg^{-1}, was injected at 0 min to prevent the effect of endogenous adrenaline released from adrenal medulla by 5-HT. ○—○, normal serum; ●—●, anti-glucagon antiserum. Number of animals, vertical lines, ordinate, and abscissa as for Fig. 4.

DISCUSSION

Injection of 5-HT into rats raises blood concentrations of cAMP and glucose. But adrenaline-induced increases in cAMP and glucose are explained by cascade reactions (Lehninger 1975). 5-HT is known to stimulate the release of adrenaline from the adrenal medulla (Kobayashi et al 1960), thus, it is thought that the increase in blood concentration of glucose and cAMP by 5-HT is the result of it releasing adrenaline from the adrenal medulla. In brown adipose tissue, in vitro, the effect of 5-HT on lipolysis is due to it releasing noradrenaline from nerve ends in the tissue (Steiner & Evans 1976). We also had evidence that suggested the indirectness of the 5-HT effect on lipolysis in rat brown adipose tissue (Itaya 1978a).

If the effect of 5-HT on blood glucose and cAMP results from its causing release of adrenaline from the adrenal medulla, a block of the adrenaline effect on glucose and cAMP should diminish the effect of 5-HT on both parameters. However, propranolol, hexamethonium and cyproheptadine did not diminish the effect of 5-HT on cAMP, but affected 5-HT's action on glucose concentrations. These findings together with the inability of atropine to modify the effect of 5-HT on either parameter suggest that the action of 5-HT on cAMP is not mediated via adrenergic or cholinergic nerves, rather it is the result of some other mechanism.

Although the effect of 5-HT on plasma cAMP is independent of that on blood glucose, 5-HT antagonists should inhibit the effects of 5-HT on both parameters, but cyproheptadine did not inhibit the effect of 5-HT on plasma cAMP, only that on glucose (Fig. 3), and did not modify the effect of adrenaline which suggests that adrenaline released from the adrenal medulla by 5-HT increases glucose in blood, and that cyproheptadine antagonizes this action of 5-HT. The increase in plasma cAMP by 5-HT seems to be mediated by a factor or factors released from the adrenal medulla by 5-HT because the action of 5-HT was diminished in adrenomedullectomized rats (Fig. 4). However, the mechanism by which this occurs is complex because the action of factors released from adrenal medulla also seem to be mediated via glucagon release.

5-HT does not directly stimulate the release of glucagon in vivo, although glucagon elevates plasma cAMP (Kunitada et al 1978). Fig. 4 also shows that the adrenal medulla is necessary for the action of 5-HT in elevating plasma cAMP. 5-HT at 4 mg kg^{-1} increases blood pressure, which will increase plasma cAMP but the increase was inhibited by anti-glucagon antiserum which suggests that the 5-HT-induced increase in plasma cAMP is not so mediated. We feel our evidence supports a hypothesis that 5-HT stimulates the release of a factor or factors from adrenal medulla and then glucagon released by the factor or factors increases the concentration of plasma cAMP.

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