in-vivo. Until now, most researchers have assumed that the concentration of 6-oxo-PGE_1 in plasma required to exert a threshold platelet anti-aggregatory effect in man is ca 1 ng ml^{-1}. This is based on experiments in which the anti-aggregatory potency of this prostaglandin was assessed in PRP prepared from man and animals. In the light of the present results, it is clear that much lower concentrations of 6-oxo-PGE_1 prevent platelet aggregation in human blood, a medium that must be considered a better indication of platelet function in-vivo than experiments using PRP. In our hands, even concentrations of 6-oxo-PGE_1 as low as 200 pg ml^{-1} significantly inhibited (by 17–28%) ADP-induced human platelet aggregation.

Even though 6-oxo-PGE_1 prevents platelet aggregation in human blood at subnanogram concentrations, it is unlikely that this prostaglandin exerts a significant effect on platelet function in healthy subjects in-vivo since human plasma contains less than 30 pg ml^{-1} 6-oxo-PGE_1 (Jackson et al 1982). However, we should not ignore the possibility that higher concentrations of this prostaglandin (perhaps sufficient to prevent platelet aggregation) do occur locally in the vicinity of a platelet plug formed from PGI_2 and/or 6-oxo-$PGF_{1\alpha}$ by platelet cytoplasmic 9-PGDH. Furthermore, elevated plasma 6-oxo-PGE_1 has been observed in patients with Barrter's syndrome and may be responsible for the defect in platelet aggregation and increased bleeding time which characterizes this condition. We suggest that the results of the present study be borne in mind when interpreting the relevance of plasma 6-oxo-PGE_1 levels in healthy human volunteers and in patients with clinical disease.

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REFERENCES

Griffiths, R. J., Lofts, F. J., Moore, P. K. (1982) Br. J. Pharmacol. 77: 491P

Griffiths, R. J., Moore, P. K. (1983) J. Pharm. Pharmacol. 35: 184–186

Jackson, E. K., Merzer, W. A., Zimmerman, J. B., Branch, R. A., Oates, J. A., Gerkens, J. F. (1981) J. Pharmacol. Exp. Ther. 216: 24–27

Jackson, E. K., Goodwin, R. P., Fitzgerald, G. A., Oates, J. A., Branch, R. A. (1982) Ibid. 221: 183–187

Korbut, R., Byrska-Danek, A., Gryglewski, R. J. (1983) Thromb. Haemostas. 50: 893

Kury, P. G., Ramwell, P. W., McConnell, H. M. (1974) Biochem. Biophys. Res. Commun. 56: 478–483

Miller, O. V., Aiken, J. W., Shebuski, R. J., Gorman, R. R. (1980) Prostaglandins 20: 391–400

Moore, P. K. (1979) Thromb. Haemostas. 47: 76

Moore, P. K., Griffiths, R. J. (1983) Prostaglandins 26: 509–517

Nelson, P. K., Brookins, J., Fisher, J. W. (1983) J. Pharmacol. Exp. Ther. 226: 493–499

Schwertschlag, U., Stahl, T., Hackenthal, E. (1982) Prostaglandins 23: 129–138

Spannhake, E. W., Levin, G. L., Hyman, A. L., Kadowitz, P. J. (1981) Prostaglandins 21: 266–275

Wong, P. Y.-K., McGiff, J. C., Sun, F. F., Lee, W. H. (1979) Eur. J. Pharmacol. 60: 245–248

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Cocaine-like action of diphenhydramine in cat cerebral arteries

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Diphenhydramine ($5{\cdot}3 \times 10^{-7}$ M) significantly reduced the tritium efflux evoked by 10^{-7} M tyramine from cat cerebral arteries preloaded with [^{3}H]noradrenaline but not that brought about by 50 mM KCl. These results indicate the ability of diphenhydramine to block the amine neuronal uptake.

Histamine has the property of releasing noradrenaline from sympathetic nerve endings present in the walls of cat and human cerebral arteries (Marco et al 1980; Balfagón et al 1984; Marco et al 1984). Most of the available data suggest that it achieves this effect by means of an exocytotic process after entering the nerve terminals through the amine uptake system (Balfagón et al 1984). This action of histamine shows a strong dependence on external calcium and appears inhibited in the presence of cocaine or colchicine. Nevertheless, such a conclusion seems to be obscured by the fact that diphenhydramine also blocks the tritium release induced by histamine from cat cerebral arteries preloaded with [^{3}H]noradrenaline, which would indicate the possible activation of a presynaptic receptor by this amine (Marco et al 1980). In the present communication we try to elucidate the way in which diphenhydramine is able to interfere with the release of noradrenaline evoked by histamine in this kind of vessel.

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Methods

Cats of either sex, 1·5–4 kg, were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.) and killed by bleeding. The brain was removed and the circle of Willis with its branches was dissected out. The vessels were cleaned to remove traces of blood and surrounding tissue and incubated for 1 h in Krebs-Henseleit solution

containing [^{3}H]noradrenaline (Amersham, 2 μCi ml^{-1}, spec. act. 10·5 Ci mmol^{-1}), which was continuously bubbled with a 95% O_2–5% CO_2 mixture and kept at 37 °C. After this incubation period the arteries were transferred into a superfusion chamber maintained at 37 °C through which prewarmed Krebs-Henseleit solution flowed at a constant rate of 0·5 ml min^{-1} by means of a perfusion pump and was aerated with 95% O_2–5% CO_2. Once the spontaneous tritium content of the effluent had reached a steady level, samples were collected every 3 min. Aliquots, 0·5 ml, were added to vials containing 10 ml of Bray's solution, the radioactivity being measured in a scintillation counter.

After 9 min of collecting effluent, a second pump delivered Krebs-Henseleit solution containing KCl or tyramine at a flow rate of 0·05 ml min^{-1} and remained on for 12 min. To study the effect of diphenhydramine on the tritium release evoked by these chemicals, the Krebs-Henseleit solution of the first pump was quickly replaced by another with the drug after 9 min of collecting perfusate and 9 min before the infusion of the releasing agents into the chamber. Diphenhydramine was present in the chamber throughout the rest of the experiment.

Results and discussion

The two hypotheses tested in the present work regarding the mechanism of action of diphenhydramine were that it could impair either the exocytotic process or the amine neuronal uptake. Since 5·3 × 10^{-7} M diphenhydramine reduced significantly the increase of tritium outflow elicited by 10^{-7} M tyramine (Fig. 2) but not that evoked by 50 mM KCl (Fig. 1), one may assume that the antihistamine has the property of blocking the entry of amines in the sympathetic nerve endings in the same way as cocaine. The effect of this concentration of diphenhydramine lies within the inhibition obtained with 10^{-6} and 10^{-7} M cocaine (Fig. 2). This cocaine-like action of antihistamines was described previously in hearts from rat and guinea-pig and mouse mast cells (Isaac & Goth 1965; Fantozzi et al 1975) and would explain the inhibition of the indirect adrenergic effect of histamine by diphenhydramine. Since histamine needs to enter the sympathetic nerve ending through the amine uptake system to release noradrenaline (Marco et al 1980), diphenhydramine would reduce this by means of inhibiting the access of histamine into the nerve terminal.

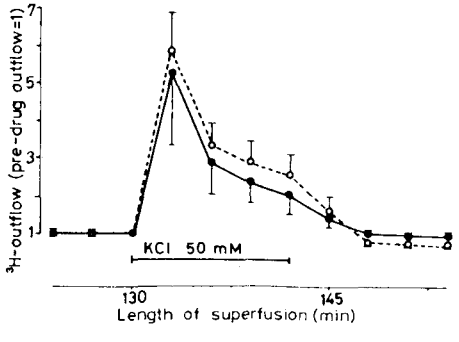

FIG. 1. Effect of 5·3 × 10^{-7} M diphenhydramine (○—○) (n = 4) on the tritium efflux evoked by 50 mM KCl from cat cerebral arteries. Control ●—● (n = 4). Each point represents the mean ± s.e.m.

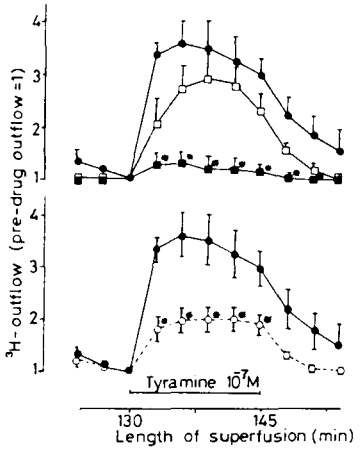

FIG. 2. Effect of 5·3 × 10^{-7} M diphenhydramine (○—○) (n = 5) (lower plot) and cocaine (□—□ 10^{-7}, ■—■ 10^{-6} M) (upper plot) on the tritium efflux evoked by 10^{-7} M tyramine from cat cerebral arteries. Control ●—● (n = 5). Each point represents the mean ± s.e.m. * Denote statistically significant differences.

If the indirect adrenergic mechanism of histamine is shown in the future to be not only restricted to cerebral blood vessels but to be present in other tissues, it should be borne in mind that a reduction in the overall response of the organ to histamine by the action of antihistamines would less likely to be due to the blockade of a histamine receptor than to the inhibition of its uptake by the nerve ending.

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REFERENCES

Balfagón, G., Galván, R., Marco, E. J. (1984) J. Pharm. Pharmacol. 36: 248–252

Fantozzi, R., Franconi, F., Mannaioni, P. F., Masini, E., Moroni, F. (1975) Br. J. Pharmacol. 53: 569–574

Isaac, L., Goth, A. (1965) Life Sci. 4: 1899–1904

Marco, E. J., Balfagón, G., Marín, J., Gómez, B., Lluch, S. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 312: 239–243

Marco, E. J., Balfagón, G., Conde, M. V. (1984) J. Pharm. Pharmacol. 36: 253–255