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Interactions of bradykinin, prostaglandin E_1, 5-hydroxytryptamine, histamine and adenosine-5′-triphosphate on the dye leakage response in rat skin

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Dye leakage in rats produced by intracutaneous injections of irritants was quantitated using an azovan blue technique. Concentration-response lines were obtained for irritants alone and in presence of constant concentrations of other irritants. 5-Hydroxytryptamine (5-HT) (10^{-7}M) potentiated histamine, bradykinin and prostaglandin E_1 (PGE_1), but inhibited adenosine-5′-triphosphate (ATP). Histamine (10^{-5}M) potentiated bradykinin, PGE_1 and ATP. Bradykinin (10^{-6}M) potentiated PGE_1 only, but PGE_1 (10^{-6}M) potentiated bradykinin, 5-HT and histamine. ATP (10^{-4}M) potentiated 5-HT, histamine and PGE_1. Release of ATP during stimulation of sensory nerves might explain enhanced neurogenic oedema observed in skin surrounding an area pretreated with compound 48/80.

In anaesthetized rats pretreated with intracutaneous injections of compound 48/80, neurogenic oedema induced by electrical stimulation of the saphenous nerve (Chahl & Ladd, 1976) or by capsaicin or formalin (Arvier, Chahl & Ladd, 1976 submitted for publication) was reduced in the pretreated area but enhanced in the surrounding area. Prostaglandins of the E-type have been shown to produce potentiation of the inflammatory responses to bradykinin and histamine (Moncada, Ferreira & Vane, 1973; Williams & Morley, 1973; Bekemeier, Giessler & Hirschelmann, 1974). These observations prompted this study where the interactions have been examined of some mediators of inflammation, with each other, and with adenosine-5′-triphosphate, which has been postulated to be involved in antidromic vasodilatation (Kiernan, 1972b).

METHODS

Male rats, Wistar strain, 100–150 g, were anaesthetized with ether and the tail was warmed in water at 45–50°. An injection of azovan blue solution (2·5 ml kg^{-1} of a 2% solution) was given into a lateral tail vein. Intracutaneous injections of mediators were given into separate regions of the shaved abdominal skin in 0·05 ml volumes. To obtain results for control concentration-response lines for the mediators, four concentrations were injected in a geometric progression with a factor of 2. All mediators were diluted in Tyrode solution and a control intracutaneous injection of Tyrode was given to each animal. The mediators used were 5-hydroxytryptamine (5-HT), histamine, bradykinin, prostaglandin E_1 (PGE_1) and also ATP. Where combinations were tested, the same concentrations of each substance as were given to controls were injected, but each solution also contained a constant low concentration of another mediator [5-HT (10^{-7}M); histamine (10^{-5}M); bradykinin (10^{-6}M); PGE_1 (10^{-6}M); ATP (10^{-4}M)]. A separate control injection of the constant concentration of the added mediator was also made in test rats, The effect of a constant concentration of each substance was tested on the varying responses to each other. Five animals were used for each concentration-response line and the injection sites were varied to equalize possible variance in the results due to differences in the abdominal skin sites. Animals were killed 20 min after the last injection. The abdominal skin was removed, the blue areas removed, cut fine and placed into a mixture of sodium sulphate and acetone according to the method of Harada, Takeuchi, & others, (1971). Twenty-four h later the solutions were centrifuged and the amount of dye estimated spectrophotometrically at 620 nm.

Concentration-response lines were plotted by eye as absorbance against concentration on a logarithmic scale through the mean responses at each concentration. Pairs of concentration-response lines were compared by a two-way analysis of variance with partitioning of the concentration and interaction variances into linear, quadratic and cubic components by the method of orthogonal contrasts. This allowed testing of the significance of curvilinear regression (the quadratic and cubic components) and the effect of treatment on the shape of the concentration-response lines.

Drugs. The following drugs were used: adenosine-5′-triphosphate disodium salt (Sigma); bradykinin triacetate (Sigma); Evans blue (Difco); histamine diphosphate (Sigma); 5-hydroxytryptamine creatinine sulphate (Sigma); prostaglandin E_1 (Upjohn). The composition of the Tyrode solution in g litre^{-1} was: NaCl, 8·0; KCl, 0·2; $MgCl_2$, 0·1; $CaCl_2$, 0·2; NaH_2PO_4, 0·05; $NaHCO_3$, 1·0.

RESULTS

The results are summarized in Table 1 and shown graphically in Figs 1-5. All concentration-response relations were fitted adequately by a straight line since there was no significant quadratic or cubic component of the concentration variance of the two-way analyses of variance. The control responses to the mediators shown in each graph were obtained in the same week as the responses to mediators in the presence of a constant concentration of another mediator. The control concentration-response line to 5-HT shown in Fig. 4 was obtained in the summer whereas that shown in the other figures was obtained in the late winter. A seasonal variation in the dye leakage response to 5-HT in rats was also found by Arvier, Chahl & Ladd (1976, submitted for publication). PGE_1 alone produced very little response in these experiments which were made in winter. Bradykinin responses were usually poorly related to concentration.

Degrees of potentiation, on an arbitrary scale, and significance levels of potentiations are shown in Table 1. It was found that 5-HT (10^{-7}M) significantly potentiated the responses to histamine, bradykinin and PGE_1, but inhibited the responses to ATP (Table 1; Fig. 1). Histamine (10^{-5}M) potentiated the responses to bradykinin, PGE_1 and ATP but did not significantly affect the responses to 5-HT (Table 1; Fig. 2). Bradykinin (10^{-6}M) potentiated responses to PGE_1 only (Table 1; Fig. 3). PGE_1 (10^{-6}M) potentiated bradykinin, 5-HT and histamine but did not affect responses to ATP (Table 1; Fig. 4). ATP (10^{-4} M) potentiated 5-HT, histamine and PGE_1 but not bradykinin (Table 1; Fig. 5). Potentiations often appeared more marked at the higher concentrations used to obtain concentration-response lines. However, significant interactions between concentration and the effect of a potentiating mediator from two-way analysis of variance were found for histamine with PGE_1 (10^{-6}M) ($P < 0.001$), bradykinin with histamine (10^{-5}M) ($0.05 > P > 0.01$), PGE_1 with histamine (10^{-5}M) ($0.05 > P > 0.01$) and PGE_1 with ATP (10^{-4}M) ($0.05 > P > 0.01$), indicating that changes in slope of the concentration-response lines by potentiating mediators were significant for these combinations only.

Table 1. *Degree of potentiation† of dye leakage produced by constant concentrations of mediators upon concentration*(M)*-response lines to other mediators* (5-HT $6.25 \times 10^{-7} - 5 \times 10^{-6}$; histamine $5 \times 10^{-5} - 4 \times 10^{-4}$; bradykinin $1.25 \times 10^{-6} - 1 \times 10^{-5}$; PGE_1, $7.25 \times 10^{-7} - 6 \times 10^{-6}$; ATP $6.25 \times 10^{-4} - 5 \times 10^{-3}$)

Const. concn.	Concentration response line: 5-HT	Hist.	Brady.	PGE_1	ATP
5-HT (10^{-7} M)		+*	++***	+***	---***
Histamine (10^{-5} M)	0		++***	+***	+++***
Bradykinin (10^{-6} M)	0	0		+***	0
PGE_1 (10^{-6} M)	++***	+++***	++***		0
ATP (10^{-4} M)	++***	+++***	0	++***	

† Degree of potentiation on arbitrary scale: 0, none; +, slight; ++, marked; +++, very marked inhibition.
* $0.05 > P > 0.01$; **, $0.01 > P > 0.001$; ***, $P < 0.001$.
Concentrations of mediators used are shown in parentheses.

DISCUSSION

The results presented here confirm previous findings that PGE_1 potentiates responses to bradykinin and histamine (Moncada & others, 1973; Williams & Morley, 1973; Bekemeier & others, 1974), and clearly demonstrate that the effects are not simply additive. All concentrations of bradykinin used appeared to be potentiated by PGE_1 to a similar degree and the bradykinin concentration-response line remained relatively flat in the presence of PGE_1. Bradykinin and PGE_1 were mutually potentiating since PGE_1 was potentiated by bradykinin. The most remarkable potentiation found was that of histamine by PGE_1, particularly at the higher concentrations of histamine used. In view of the magnitude of these potentiated histamine responses, albeit at relatively high histamine concentrations, a major role for histamine in oedema production during the acute inflammatory response cannot be dismissed. PGE_1 also potentiated 5-HT which is present in rat mast cells (Benditt, Wong & others, 1955) and plays an important role in oedema production in inflammatory reactions in this species (Parratt & West, 1958). However Lewis, Nelson & Sugrue (1975) did not find potentiation by PGE_1 of rat hind paw oedema induced by histamine or 5-HT.

Both histamine and 5-HT potentiated bradykinin and PGE_1, which raises the possibility that low concentrations of the amines might play a greater

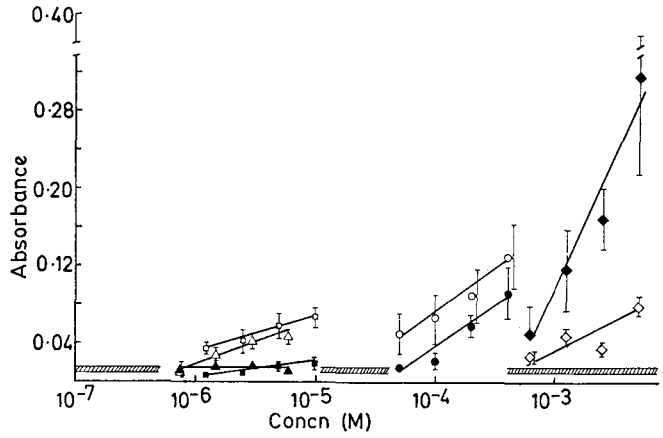

FIG. 1. Effect of 5-HT (10^{-7} M) on the concentration-response lines of histamine, bradykinin, PGE_1 and ATP. Lines are plotted by eye as absorbance against concentration (M) on a logarithmic scale. Each point represents the mean of 5 responses. Vertical bars represent standard errors of the means. Mean histamine responses are shown as circles, ATP responses as diamonds, bradykinin responses as squares and PGE_1 responses as triangles. Mean control responses are shown as closed symbols and responses in the presence of 5-HT as open symbols. The cross-hatched area represents the mean ± standard error of 20 responses to 5-HT (10^{-7} M).

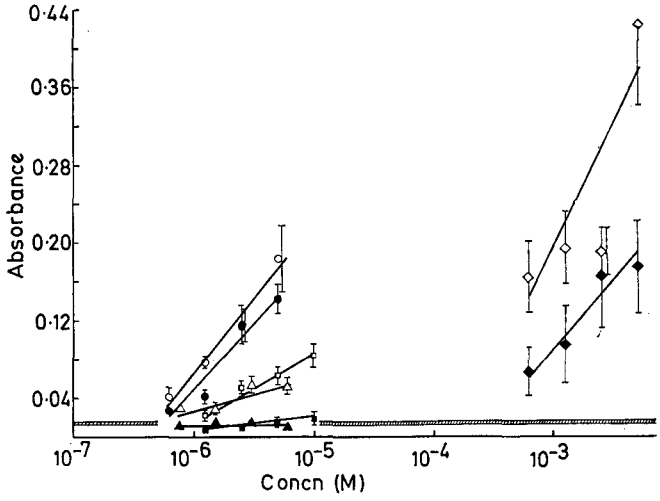

FIG. 2. Effect of histamine (10^{-5} M) on the concentration-response lines to 5-HT, bradykinin, PGE_1 and ATP. See Fig. 1. Mean 5-HT responses are shown as circles, ATP responses as diamonds, bradykinin responses as squares and PGE_1 responses as triangles. Mean control responses are shown as closed symbols and responses in the presence of histamine as open symbols. The cross-hatched area represents the mean ± standard error of 20 responses to histamine (10^{-5} M).

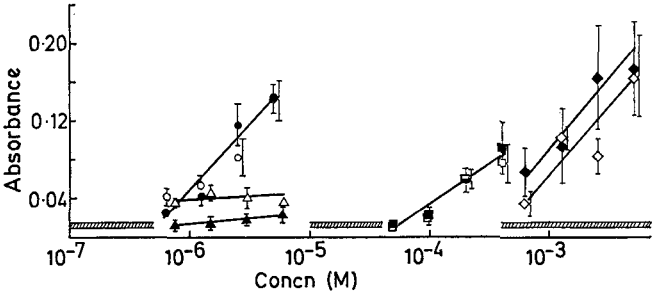

FIG. 3. Effect of bradykinin (10^{-6} M) on the concentration-response lines to 5-HT, histamine, PGE_1 and ATP. See Fig. 1. Mean 5-HT, responses are shown as circles, ATP responses as diamonds, histamine responses as squares and PGE_1 responses as triangles. Mean control responses are shown as closed symbols and responses in the presence of bradykinin as open symbols. The cross-hatched area represents the mean ± standard error of 15 responses to bradykinin (10^{-6} M).

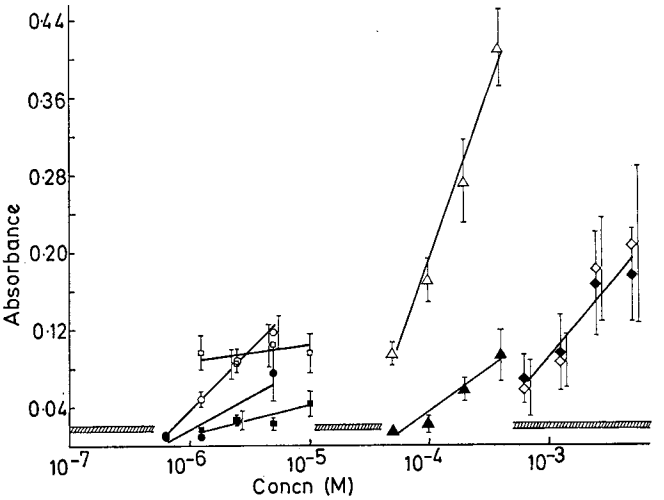

FIG. 4. Effect of PGE_1 (10^{-6} M) on the concentration-response lines of 5-HT, histamine, bradykinin and ATP. See FIG. 1. Mean 5-HT responses are shown as circles, ATP responses as diamonds, histamine responses as triangles and bradykinin responses as squares. Mean control responses are shown as closed symbols and responses in the presence of PGE_1 as open symbols. The cross-hatched area represents the mean ± standard error of 15 responses to PGE_1 (10^{-6} M).

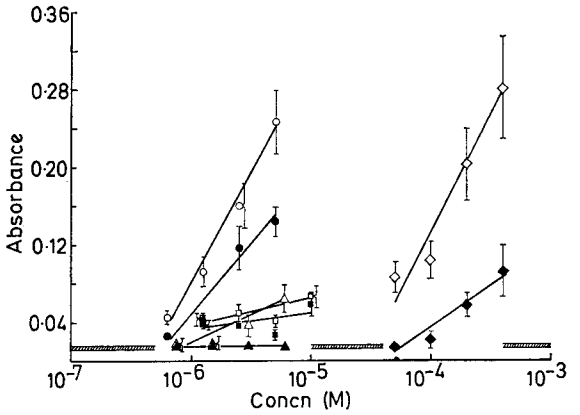

FIG. 5. Effect of ATP (10^{-4} M) on the concentration-response lines to 5-HT, histamine, bradykinin and PGE_1. See Fig. 1. Mean 5-HT responses are shown as circles, histamine responses as diamonds, bradykinin responses as squares and PGE_1 responses as triangles. Mean control responses are shown as closed symbols and responses in the presence of ATP as open symbols. The cross-hatched area represents the mean ± standard error of 20 responses to ATP (10^{-4} M).

role in the later stages of inflammation than has been previously supposed. It is unknown whether antihistamines and anti-5-HT drugs block these potentiating actions of the amines.

ATP produced potentiation of PGE_1 and 5-HT and marked potentiation of histamine. However ATP was potentiated only by histamine and was inhibited by 5-HT. Holton (1959) found that ATP was released from sensory nerves during antidromic stimulation. Since ATP has been shown to cause release of amines from mast cells (Kiernan, 1972a), Kiernan (1972b) suggested that ATP is the mediator of antidromic vasodilatation. If indeed ATP is released on electrical or chemical stimulation of sensory nerves, irrespective of whether it is the mediator of neurogenic oedema, it is possible that it might enhance the action of any histamine or 5-HT in the region, such as might be present surrounding an area pretreated with compound 48/80. This would provide an explanation for the previous observation that neurogenic oedema induced by electrical stimulation of the saphenous nerve in rats (Chahl & Ladd, 1976) or by capsaicin or formalin (Arvier, Chahl & Ladd, 1976, submitted for publication),

was suppressed in areas of skin pretreated with intracutaneous injections of compound 48/80, but enhanced in areas surrounding the pretreated areas.

Although significant interactions between several combinations of two mediators of inflammation have been observed in these experiments, the mechanisms by which these occur, or by which they may be modified, remain to be investigated. Investigation of the effects of various combinations of three or more mediators, such as might occur in pathological processes, might provide even further insight into oedema production during the inflammatory response.

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