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ATP causes postjunctional potentiation of noradrenergic contractions in the portal vein of guinea-pig and rat*

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Superfusion of the portal vein of rat and guinea-pig with Krebs' solution maintained at 25 °C greatly inhibits spontaneous contractions of the preparations and allows contractile responses to ATP and noradrenaline to be measured accurately. Under these conditions, continuous superfusion with ATP (10^{-5} M), a concentration which had no effect on either basal tension or spontaneous activity, caused a significant shift to the left of the concentration-response curve to exogeneous noradrenaline in both tissues. The mechanism of this potentiation induced by ATP may differ in the two tissues since in the rat portal vein potentiation appeared to be rapidly reversed by superfusing with ATP-free solution, whereas in the guinea-pig portal vein a further concentration-response curve to noradrenaline, in the absence of ATP, was still significantly shifted to the left compared with the control curve. However, potentiation in the rat portal vein may have had a longer duration than is suggested by the results since control concentration-response curves to noradrenaline in this tissue showed a progressive shift to the right which, although not significant, is likely to have affected the apparent time course of potentiation. It is concluded that ATP can potentiate contractions to exogeneous noradrenaline in the portal vein of rat and guinea-pig via an, as yet, unidentified postjunctional mechanism.

The portal vein of the rat and guinea-pig receives a dense adrenergic innervation (Johansson et al 1970; Burnstock et al 1979). A previous study in this laboratory showed that 2-chloroadenosine, a structural analogue of adenosine, depressed contractions to sympathetic nerve stimulation in the portal vein of the rat via prejunctional P_1-purinoceptors, but potentiated those in the portal vein of the guinea-pig via postjunctional P_1-purinoceptors (Burnstock et al 1984). ATP affects basal tension and spontaneous activity in both preparations (Sjöberg & Wahlstrom 1975; Burnstock et al 1979) and depolarizes the smooth muscle cells of the rat portal vein (Karashima & Takata 1979). We have, therefore, extended our previous experiments by examining postjunctional effects of ATP on contractions to exogenous NA. Superfusion of preparations with ATP was used to ensure that intact ATP was constantly present at the smooth muscle cell surface since the previous study (Burnstock et al 1984) indicated that ATP was rapidly broken down in the bathing solution in organ bath studies. Furthermore, the concentrations of ATP used were subthreshold for mechanical activity.

Materials and methods

Pharmacology. A section of rat and guinea-pig portal vein was removed and mounted vertically as described previously (Burnstock et al 1984). The tissues were superfused with modified Krebs' solution of the following composition (mM), NaCl 120; KCl 5; $CaCl_2$ 2·5; $MgSO_4$ 1·2; K_2HPO_4 1·2; $NaHCO_3$ 25; glucose 11; $CaNa_2$ EDTA 0·032; ascorbic acid 0·55 (pH 7·3–7·4) maintained at a temperature of 25 °C and bubbled continuously with 95% O_2/5% CO_2. A superfusion rate of 3 ml min^{-1} was maintained by a Watson-Marlow peristaltic pump. Drugs were applied to the tissue via the superfusion cannula. The following experimental protocol was used: following construction of a control concentration-response curve for NA, the tissue was superfused continuously with a concentration of ATP which was subthreshold for mechanical activity and the concentration-response curve for NA was repeated. A further concentration-response curve for NA was then produced, acting as an indication of the duration of the action of ATP.

Analysis of results. Concentration-response curves were constructed according to the method of Waud (1975). This method of combining log concentration-response curves avoids biasing the curves towards a lower slope. All concentration-response curves were analysed at the EC20, 35, 50, 65 and 80 levels using Student's paired *t*-test. A probability of less than 0·05 was considered statistically significant.

Drugs. Noradrenaline bitartrate (NA) and adenosine 5'-triphosphate (ATP) (both obtained from Sigma), were made up in modified Krebs' solution.

Results

Initial studies indicated that at 37 °C, in either conventional organ bath experiments or in superfusion experiments, both rat and guinea-pig preparations showed a high level of spontaneous activity, sometimes as great as 50% of the maximum contraction to NA, making it difficult to measure responses to NA and ATP accurately. Typical responses from two rat portal vein preparations superfused with modified Krebs' solution,

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showing both a high level of spontaneous activity and its superimposition upon contractions to NA, are seen in Fig. 1.ai, aii. Furthermore, the similarity in potency of ATP and adenosine in organ bath experiments suggested that ATP was rapidly broken down to adenosine on addition to the bathing solution. These problems could be minimized or eliminated by superfusion with modified Krebs' solution maintained at 25 °C. The size of spontaneous phasic contractions relative to tonic contractions to exogenous NA were clearly smaller when preparations were superfused at 25 °C than when at 37 °C (Fig. 1.bi, bii). Furthermore, superimposed phasic contractions on the tonic contractile response to NA were not seen at the lower temperature (Fig. 1.bi, bii).

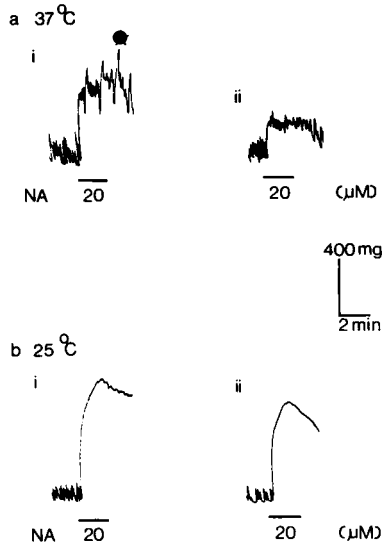

FIG. 1. Contractions of the isolated rat portal vein longitudinal muscle to noradrenaline (NA) (2×10^{-5} M) when superfused with modified Krebs' solution maintained at (ai, aii) 37 °C (bi, bii) 25 °C. Note in ai, ii the size of spontaneous contractions relative to, and their superimposition upon (starred), contractions to NA and lack of superimposition of spontaneous activity on noradrenergic contraction in bi, bii. Each trace is taken from a different preparation.

Continuous superfusion of the guinea-pig portal vein at 25 °C with ATP (10^{-5} M) had no effect on basal tension or spontaneous activity but did produce a significant potentiation of the contractile response to NA (10^{-7} to 10^{-4} M) ($P < 0.01$) (Fig. 2b). At the EC50 level a 1·7-fold shift to the left of the NA concentration-response curve was seen (control EC50, 5.4×10^{-6} M, 95% confidence limits 4.3×10^{-6} to 6.8×10^{-6} M: test EC50, 3.2×10^{-6} M, 95% confidence limits 1.9×10^{-6} to 5.1×10^{-6} M). However, a further concentration-response curve to NA was also significantly different from the initial control curve ($P < 0.001$) (EC50, 2.9×10^{-6} M, 95 % confidence limits $2.1 \times 10^{-}$-6 to 3.9×10^{-6} M) though not from that produced in the presence of ATP (Fig. 2b). Three consecutive concentration-response curves to NA obtained over the same time course as above were not significantly different from each other (Fig. 2a), indicating that the increased sensitivity to NA in the presence of ATP was not due to unrelated changes in tissue activity.

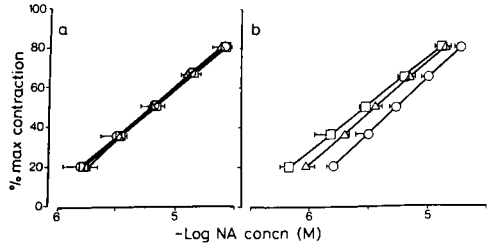

FIG. 2. Guinea-pig isolated portal vein superfused with modified Krebs' solution maintained at 25 °C. (a) Three consecutive concentration-response curves to noradrenaline (NA) (10^{-4} M). ○—initial; △—repeat; □—final curve (n = 4). (b) Three consecutive concentration-response curves to NA (10^{-7} to 10^{-4} M). ○—initial; △—repeat while tissue is continuously superfused with ATP (10^{-5} M); □—final curve in absence of ATP (10^{-5} M) (n = 6). Horizontal bars show s.e.m. at the EC 20, 35, 50, 65 and 80 levels.

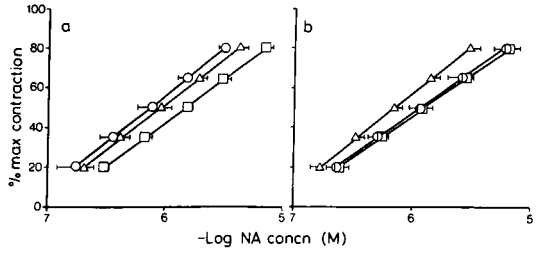

FIG. 3. Rat isolated portal vein superfused with modified Krebs' solution maintained at 25 °C. (a) Three consecutive concentration-response curves to noradrenaline (NA) (10^{-8} to 2×10^{-5} M). ○—initial; △—repeat; □—final curve (n = 4). (b) Three consecutive concentration-response curves to NA (10^{-8} to 2×10^{-5} M). ○—initial; △—repeat while tissue is continuously superfused with ATP (10^{-5} M); □—final curve in absence of ATP (10^{-5} M) (n = 7). Horizontal bars show s.e.m. at the EC 20, 35, 50, 65 and 80 levels.

Continuous superfusion of the rat portal vein at 25 °C with ATP (10^{-5} M) had no effect on spontaneous activity or basal tension. However, it did produce a statistically significant potentiation of the contractile response to NA (10^{-8} to 2×10^{-5} M) ($P < 0.001$) (Fig. 3b) with a 1·7-fold shift to the left at the EC50 level of the concentration-response curve to NA (control EC50, 1.1×10^{-6} M, 95% confidence limits 6.6×10^{-7} to 1.7×10^{-6} M: test EC50, 6.3×10^{-7} M, 95% confidence limits 4.0×10^{-7} to 1.0×10^{-6} M). A further concentration-response curve to NA in the absence of ATP was not significantly different from the initial control NA curve (EC50, 1.00×10^{-6} M, 95% confidence limits 5.6×10^{-7} to 1.8×10^{-6} M) (Fig. 3b). Three control concentration-

response curves to NA, obtained over the same time course as above, although showing a slight shift to the right over this time course, were not significantly different from each other (Fig. 3a).

Discussion

This study shows that ATP can act postjunctionally in the rat and guinea-pig portal vein to potentiate the contractile action of NA. ATP in a concentration that had no effect here on either basal tension or spontaneous activity (10^{-5} M), caused a similar shift to the left of the concentration-response curve to NA in both tissues. In the guinea-pig portal vein the potentiation had a long time course since a further concentration-response curve to NA, in the absence of ATP, was still significantly shifted to the left compared with the control. The potentiation in both preparations was not due to a non-specific change in tissue sensitivity over the course of the day since three consecutive concentration-response curves to NA, over the same time course, were not significantly different from each other. The time course of the potentiation in the rat portal vein is less clear since this effect appeared to be rapidly reversed by superfusion with ATP-free solution. However, control concentration-response curves to NA showed a progressive shift to the right which, although not significant, is likely to have affected the apparent time course of potentiation. Thus potentiation in the rat portal vein may have had a longer duration than is suggested by the results.

Adenine nucleoside and nucleotides have been shown to interact with NA postjunctionally in a variety of isolated tissues (see Stone 1983). The mechanism of these synergistic interactions between NA and purines is not clear. Several possibilities can, however, be considered. It has been suggested that purinoceptors may have a close association with α-adrenoceptors and may in some way affect the sensitivity of these α-adrenoceptors (see Stone 1983). However, potentiation may be due to a more generalized sensitizing action. Although the concentration of ATP used was subthreshold for mechanical activity it is not known whether it was subthreshold for electrical activity. The portal vein of the guinea-pig is characterized by regenerative electrical activation (Hermsmeyer 1971) and since purines have been shown to depolarize the rat portal vein (Karashima & Takata 1979), potentiation may be due to a change in the electrical properties of the smooth muscle. Alternatively, purines may affect the Ca^{2+}-pool utilized by NA. Finally, prostaglandins may be involved since purines have been shown to induce prostaglandin synthesis and release in a range of tissues (Needleman et al 1974). Prostaglandins have also been shown to modulate the actions of NA (Güllner 1983). The relevance of these or of other possible mechanisms is not presently known.

Postjunctional interactions between purines and NA have been recently reviewed (Stone 1983). They may be physiologically relevant and could play an important role in cotransmission from sympathetic nerves.

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