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The effects of P_2 purinoceptor agonists on the isolated portal vein of the guinea pig

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Abstract

UTP, ATP and several of its analogues enhanced contractions of the longitudinal smooth muscle layer of the guinea-pig portal vein. The rank order of potency was 2-methylthioATP > α,β-methyleneATP > adenosine tetraphosphate ≥ β,γ-methyleneATP ≥ ATP = UTP ≫ adenosine. Suramin (100 μM) blocked the contractile effects of 2-methylthioATP and α,β-methyleneATP, but not those of ATP and adenosine tetraphosphate. The P_1 purinoceptor antagonist, 8-phenyltheophylline (10 μM), was without effect on the response to ATP. Field stimulation (5 s trains every 100 s, 1 ms, 55 V) caused frequency-dependent contractions that were partially reduced by the noradrenergic neurone blocking drug, BW 172*C*58 (4-benzoyl-xylocholine, 10 μM), but not by suramin. α,β-MethyleneATP was more potent than β,γ-methyleneATP, UTP and adenosine tetraphosphate in partially inhibiting field stimulation-induced contractions of the portal vein; its effects, but not those of adenosine tetraphosphate, were reduced by suramin. These results indicate that the guinea-pig portal vein contains P_2 purinoceptors; these include a P_{2x} subtype, mediating contraction.

Keywords: P_{2x} purinoceptor; ATP; Suramin; Portal vein, guinea-pig

1. Introduction

The hepatic portal vein plays an important role in regulating blood flow to the liver. Its contractility can be modulated by adenosine and ATP, possibly derived from the dense sympathetic innervation (Burnstock et al., 1984; Chau et al., 1990).

In this study we describe the effects of adenosine, ATP, UTP and some ATP analogues on the contractile force of the longitudinally-arranged smooth muscle layer of the guinea-pig portal vein. The guinea-pig portal vein contains both circular and longitudinal smooth muscle layers, both of which exhibit spontaneous contractile activity (Burnstock et al., 1979). Recent autoradiographic studies by Bo and Burnstock (1993) have indicated that P_{2x} purinoceptors are present in both layers with the higher density being in the longitudinal layer.

Purinoceptors comprise two classes, P_1 and P_2, which are adenosine- and ATP-preferring, respectively (Burnstock, 1978; Fredholm et al., 1994). P_2 purinoceptors, in contrast to the P_1 purinoceptors of the A_1 and A_2-subtypes, are insensitive to blockade by alkyl xanthines, and can be subdivided into several subtypes, two of which (P_{2x} and P_{2y}) have been shown to play major roles in regulating the tone of vascular smooth muscle (Pearson and Gordon, 1989; Ralevic and Burnstock, 1991; Fredholm et al., 1994). These two subtypes have traditionally been distinguished on the basis of the orders of agonist potencies of a series of phosphothioate analogues of ATP, and by susceptibility to desensitization by repeated application of α,β-methylene ATP (P_{2x}) or to blockade by reactive blue 2 (P_{2y}) (Burnstock and Warland, 1987). The trypanocidal drug, suramin, blocks the actions of some analogues of ATP at both P_{2x} and P_{2y} purinoceptors (Dunn and Blakeley, 1988; Leff et al., 1990; Hoyle et al., 1990). It has also been proposed that some tissues may possess UTP-preferring pyrimidinoceptors. These receptors seem to be distinct from P_2- or nucleotide-receptors (Von Kügelgen et al., 1987; Von Kügelgen and Starke, 1990; Saiag et al., 1992).

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While it is well-documented that adenosine and ATP can affect the contractile force of the smooth muscle of the portal vein of several species (Burnstock et al., 1979, 1984; Enero, 1981; Kennedy and Burnstock, 1985; Chau et al., 1990; Bo and Burnstock, 1993; Chin et al., 1995), the nature of the purinoceptor mediating these effects in the guinea-pig portal vein has not been extensively investigated.

In addition to examining the purinoceptors mediating the contractile effects of exogenous addition of purinoceptor agonists on the longitudinal layer of the guinea-pig portal vein, we have also investigated the effects of purinoceptor agonists and antagonists, a noradrenergic neurone blocking drug and atropine on contractile responses to field stimulation of nerve terminals within the portal vein.

2. Materials and methods

2.1. Animals

Female virgin Dunkin–Hartley guinea pigs (400–700 g) were housed in open runs at 22 ± 1°C, with a 12 h light cycle. Food consisted of Clark–King laboratory pellets, green vegetables and water ad libitum. Animals were examined daily, to ascertain the state of the vaginal closure membrane, and used 7–9 days after vaginal opening (i.e. during dioestrus; Farrar et al., 1980).

2.2. Preparation of portal veins

Animals were killed by cervical dislocation, the portal veins removed and placed into a petri dish containing modified Krebs' solution (composition, mM: NaCl 118.0; KCl 4.70; $MgSO_4$ 0.45; $CaCl_2$ 2.50; glucose 11.66; KH_2PO_4 1.18; $NaHCO_3$ 25.0). A wire was inserted into the lumen of the excised portal vein to remove endothelium and to enable it to be cut, longitudinally, into two strips.

Preparations were attached to tissue holders incorporating stimulating electrodes, to permit field stimulation, and set up so as to enable the recording of isometric force developed by the outer longitudinally arranged smooth muscle layer. This was measured using Grass FTO3 transducers coupled to Grass model 79C and 79E polygraphs. Tissues were suspended under 0.75 g resting force in isolated organ baths containing modified Krebs' solution at 37°C (bubbled with 5% CO_2 in O_2; pH 7.4) and allowed to equilibrate for 60 min. Stimulation was applied using Grass S48 stimulators.

2.3. Effects of agonists in the absence and presence of antagonists

In experiments in which the effects of purinoceptor agonists and UTP were examined, tissues were field stimulated at 100 s intervals with 5 s trains of pulses (1 ms, 25 Hz, 55 V). Discrete log concentration–response curves were constructed to UTP, ATP, adenosine tetraphosphate, and the stable analogues of ATP, namely 2-methylthioATP, β,γ-methyleneATP and α,β-methylene ATP. Each concentration of each agonist was added to a preparation of portal vein, left for 200 s, then preparations were washed with fresh buffer (2 × bath vol.). Fifteen minutes later the next concentration of agonist was added.

In some experiments either the P_1 purinoceptor antagonist, 8-phenyltheophylline (10 μM), or the P_2 purinoceptor antagonist, suramin (100 μM), was added to the tissue baths and the preparations allowed to equilibrate for a minimum of 30 and 60 min, respectively, prior to the addition of agonists. After each concentration of agonist was washed out the antagonist was replaced and 15 min later the next concentration of agonist added.

In a separate series of experiments the effects of repeated application of α,β-methylene ATP on responses to adenosine tetraphosphate (10 μM) and UTP (10 μM) were examined. α,β-methyleneATP (10 μM) was added to the bath three times with washes between each addition. Responses to UTP and adenosine tetraphosphate were compared with those obtained prior to desensitization. In some experiments, one preparation from each portal vein acted as a control in that α,β-methyleneATP application was omitted.

2.4. Frequency response curves

In paired preparations from a group of guinea pigs, three frequency response curves to field stimulation (5 s trains every 100 s; 1 ms duration; 55 V; 5, 15, 25, 40 and 55 Hz) were constructed. Verification that the stimulation was of nerve terminals rather than of smooth muscle was undertaken using tetrodotoxin (1 μM). In one preparation from each animal the P_2 purinoceptor antagonist, suramin (Dunn and Blakeley, 1988; 100 μM), was present for 60 min before construction of the first frequency response curve and remained present throughout the remainder of the experiment, whereas in the other preparation from each animal it was omitted. In both groups of tissues peak heights of responses were expressed as percentages of the response to noradrenaline (100 μM) which was added at the end of each curve. One frequency-response curve was constructed, then the noradrenergic neurone blocking drug, 4-benzoylxylocholine (BW 172*C*58;10 μM; Boura et al., 1960), was added and a second curve constructed, to determine the noradrenergic contribution to the response to field-stimulation. A third curve was then constructed in the continued presence of BW 172*C*58, and in addition, atropine (10 μM), to determine whether there was a cholinergic component to stimulation. Each antagonist was allowed to equilibrate with the tissue for 30 min prior to the

construction of a frequency-response curve. Time control experiments indicated that these were highly reproducible.

2.5. Light histology

Sections of portal vein were removed from animals and placed in Tissue-Tek (Miles). Tissues were stored at −80°C prior to cutting (Reichert–Jung Cryocut E); sections (10 μm thick) were placed on gelatin-coated slides and stained with haematoxylin and eosin (Junqueira et al., 1989).

2.6. Analysis of concentration–response data

Since preparations of portal vein exhibit spontaneous contractile activity, the effects of agonists on spontaneous contractile activity were determined by recording the area under the force–time curve over 100 s (AUC) before and after the addition of agonists. AUC was measured using a digitising tablet and Sigma Scan (Jandel Scientific). The AUC in the presence of agonist, expressed as a percentage of the pre-agonist AUC, was taken as the measure of agonist activity.

The effects of agonists on responses to field stimulation were measured by determining the minimum response to field stimulation that occurred in the presence of agonist, and expressing it as a percentage of the mean of the two pre-agonist responses to field stimulation.

Mean log concentration–response curves were constructed by pooling data from individual log concentration–response curves. Linear regression analysis of points falling between 15 and 85% of maximum response, (or over the linear region where maximum response was not obtained) and comparison of lines so determined, were undertaken as described in Documenta Geigy (1970). In brief, when there was a significant regression of response with increasing concentrations of agonists, least squares regression lines were fitted to the linear portions of mean log concentration–response curves, and analysis of variance undertaken to determine deviation from parallelism and coincidence. If lines were parallel, estimates of potency ratio, together with 95% confidence limits, were determined for each analogue compared to ATP. A significant difference in apparent potency of the analogue and ATP was indicated when the 95% confidence limits of their potency ratio did not include one.

Other statistical analysis was performed by use of the standard formulae for calculating mean and S.E.M. Student's paired and non-paired t-tests were used to evaluate the significance of differences between mean values. The criterion of statistical significance applied was $P < 0.05$.

2.7. Drugs

Drugs used were from the following sources: Adenosine (Sigma) adenosine 5′-triphosphate Na_2 salt (ATP, Sigma); adenosine 5′-tetraphosphate Ba_2 salt (Sigma); α,β-methyleneATP Li salt (Research Biochemicals); β,γ-methyleneATP Na salt (Research Biochemicals); 2-methylthio ATP Na_4 (2-methylthioATP, Research Biochemicals); 4-benzoylxylocholine (BW 172*C*58, Burroughs Wellcome, gift of Professor A.L.A. Boura); (−) noradrenaline bitartrate (Sigma); suramin (a gift from Bayer, Australia); tetrodotoxin (Sigma); uridine 5′-triphosphate Na_3 salt (UTP, Calbiochem); 8-phenyltheophylline (Sigma).

Adenosine and UTP were dissolved in dimethylsulfoxide: H_2O (30:70), adenosine tetraphosphate was dissolved in 0.05 M HCl and made up to volume in modified Krebs' solution. 8-Phenyltheophylline was dissolved in 0.1 M NaOH/methanol (20:80) and diluted in Krebs' solution. Noradrenaline was dissolved in a diluent of the following composition (mM): NaCl 154.0, NaH_2PO_4 1.0 and ascorbic acid 0.23, and diluted in Krebs' solution immediately before use. All other drugs were made up in modified Krebs' solution on the day of use. All drugs except adenosine and suramin were kept on ice.

3. Results

3.1. Histological examination

Histological examination of three tissues indicated that preparations of portal vein were endothelium-denuded.

3.2. Effects of agonists on contractile activity

All tissues were spontaneously active and responded to field stimulation (5 s train, 1 ms, 55 V at 100 s) with brief, reproducible contractions superimposed on the spontaneous contractile activity. Tetrodoxin (1 μM) abolished these contractions ($n = 3$). Typical traces are shown in Fig. 1, which also illustrates the stimulant effects of ATP and α,β-methyleneATP. In control preparations both agonists produced concentration-related sustained increases in tone. Log concentration–response curves to these and the other purinoceptor agonists are shown in Fig. 2. UTP, adenosine tetraphosphate, 2-methylthioATP, α,β-methyleneATP and β,γ-methyleneATP all elicited contractile responses from this preparation. In some cases (ATP, adenosine tetraphosphate, α,β-methyleneATP) maximum responses were not elicited in the concentration ranges employed. Ratios of the apparent potencies of these agonists, relative to the potency of ATP, are shown in Table 1. The rank order of potency was 2-methylthioATP > α,β-methyleneATP > adenosine tetraphosphate ≥ β,γ-methyleneATP ≥ ATP = UTP ≫ adenosine.

The P_1 purinoceptor antagonist, 8-phenyltheophylline (10 μM), was found to be without effect on the responses to ATP (Student's paired t-test, $P > 0.05$, $n = 5$; data not shown).

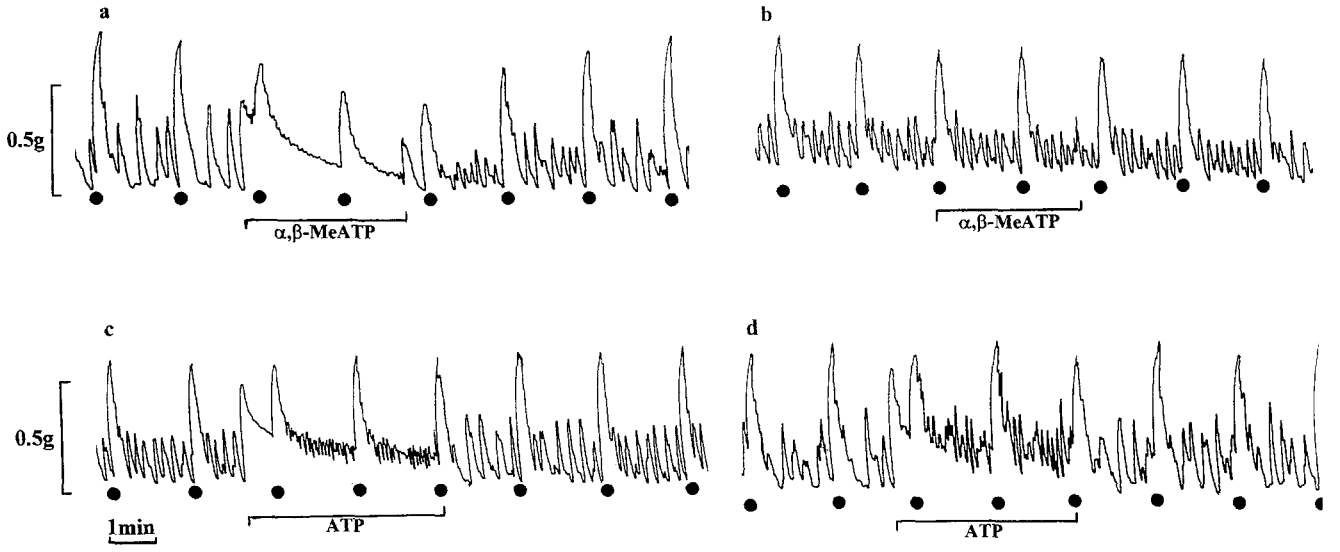

Fig. 1. Typical traces showing responses to α,β-methylene ATP (300 nM; upper panels) and ATP (100 μM; lower panels) in the absence (panels a and c) and presence (panels b and d) of suramin (100 μM). Agonists were present during the period indicated by the bar below each trace. Field stimulation (5 s trains of pulses, 1 ms, 25 Hz, 55 V at 100 s intervals) is indicated by ●.

Suramin (100 μM) produced a 27.9-fold parallel rightward shift (95% confidence limits 11.1, 82.0; df = 47) in the log concentration–response curve to 2-methylthioATP (Fig. 3, panel a) and it reduced responses to α,β-methyleneATP (Fig. 1). In contrast, it did not inhibit contractile activity elicited by adenosine tetraphosphate (Fig. 3, panel b) or ATP ($n = 5$, see Fig. 1 for a typical trace).

Repeated application of α,β-methyleneATP (10 μM), which led to tachyphylaxis to its stimulant effect, was without effect on the magnitude of responses to field stimulation ($n = 4$) or to noradrenaline ($n = 2$). In a separate series of experiments it was also without effect upon contractile responses to adenosine tetraphosphate or UTP. Thus the increase in AUC in response to UTP (100 μM) prior to the application of α,β-methyleneATP was 159 ± 10% of pre-agonist AUC, and that afterwards was 151 ± 28% (Student's paired t-test; $P > 0.05$; $n = 10$). Corresponding responses to adenosine tetraphosphate (10 μM) were 109 ± 3% and 103 ± 2%, respectively (Student's paired t-test; $P > 0.05$; $n = 4$).

3.3. Responses to field stimulation

Increases in the frequency of stimulation led to increases in the magnitude of these contractions (Fig. 4).

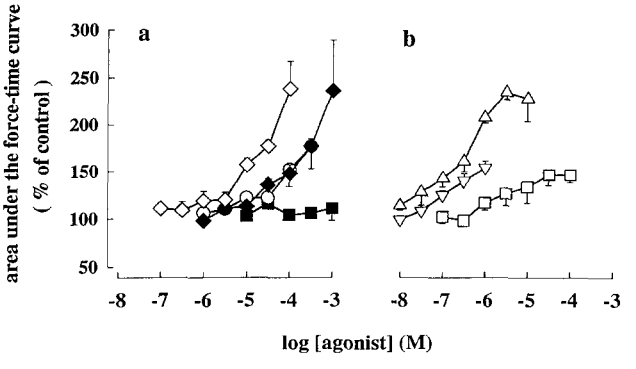

Fig. 2. Mean log concentration–response curves to agonists in preparations of field stimulated (5 s trains, 1 ms, 25 Hz, 55 V, at 100 s intervals) guinea-pig portal vein. Responses to ATP (◆), adenosine tetraphosphate (◇), UTP (○) and adenosine (■) are shown in panel a. Responses to 2methylthio-ATP (△), α,β-methylene ATP (▽) and β,γ-methylene ATP (□) are shown in panel b. The vertical axes show the area under the force time curve (AUC, g min, measured over 100 s) in the presence of agonist expressed as a percentage of the pre-agonist AUC. The horizontal axes show the log molar concentration of agonist. In all cases the points represent the mean ± S.E.M. of 5–6 observations.

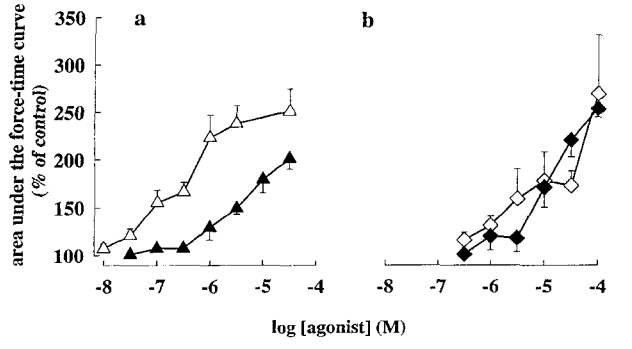

Fig. 3. The effects of suramin (100 μM) on log concentration–response curves to (a) 2-methylthioATP and (b) adenosine tetraphosphate. The filled symbols indicate agonist effects in the presence of suramin. The vertical axes show the area under the force time curve (AUC, g min, measured over 100 s) in the presence of agonist expressed as a percentage of the pre-agonist AUC. The horizontal axes show the log molar concentration of agonist. In all cases the points represent the mean ± S.E.M. of 5–6 observations.

Table 1
Potency ratios, relative to ATP, of agonists in producing contractions of guinea-pig portal vein

	Apparent potency ratio	95% confidence limits [a]
2-methylthioATP	540.8 [b]	293.4, 1075
α,β-methyleneATP	126.5	64.1, 234.3
β,γ-methyleneATP	≈ 3.0	not parallel [c]
Adenosine tetraphosphate	15.3	6.8, 36.5
UTP	1.2	0.5, 2.78
Adenosine	≪ 1	not parallel [c]

[a] Degrees of freedom = 47–83.
[b] Upper portions of lines compared; see Fig. 2.
[c] Not parallel indicates that the log concentration–response curve for the agonist and for ATP were not parallel.

There were no significant differences in the magnitudes of field stimulation-induced responses in the absence and presence of suramin (Figs. 1 and 4). The addition of the noradrenergic neurone blocking drug, BW 172*C*58 (10 μM), to preparations in the absence and presence of suramin (Fig. 4) reduced the responses to field stimulation (Student's paired *t*-tests, $P < 0.05$; $n = 5$). The subsequent addition of atropine (10 μM) was without effect ($n = 5$).

α,β-methyleneATP, and to a lesser extent β,γ-methyleneATP, adenosine tetraphosphate and UTP, inhibited responses to field stimulation (Fig. 5). The extent of the inhibition to the highest concentrations of α,β-methyleneATP used in these experiments (1 μM) was, however, less than 30% of the response to field stimulation. The effects of α,β-methyleneATP were abolished after its repeated application. 2-MethylthioATP, ATP (Fig. 5) and adenosine ($n = 6$; data not shown) were ineffective in inhibiting responses to field stimulation.

Suramin (100 μM) produced a 21.7-fold rightward shift (95% confidence limits: 6.1, 166.1; df = 31) in the log concentration–response curve for the neuro-inhibitory effects of α,β-methyleneATP (Fig. 1a, b, Fig. 6, panel a), but did not affect the inhibition of field stimulation-induced contractions elicited by adenosine tetraphosphate (potency ratio: 2.09; 95% confidence limits 0.68, 9.8; df = 42; Fig. 6, panel b).

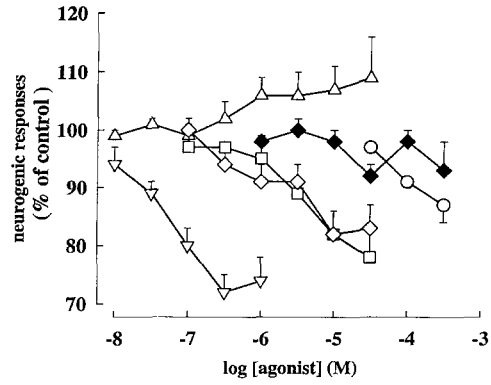

Fig. 5. The effects of agonists on responses to field stimulation (5 s trains, 1 ms, 25 Hz, 55 V, at 100 s intervals) in preparations of guinea-pig portal vein. Agonists were 2-methylthioATP (△), α,β-methylene ATP (▽), β,γ-methylene ATP (□), adenosine tetraphosphate (◇), ATP (◆) and UTP (○).The vertical axis shows responses to field stimulation in the presence of agonist expressed as a percentage of pre-agonist responses to field stimulation. The horizontal axis show the log molar concentration of agonists. In all cases the points represent the mean ± S.E.M. of 5–6 observations.

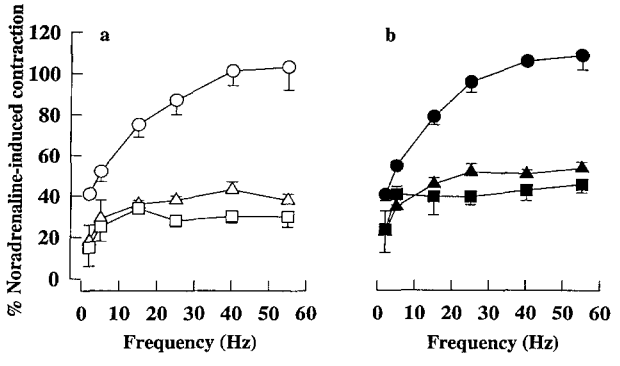

Fig. 4. Frequency response curves to field stimulation of intrinsic nerve terminals of guinea-pig portal vein. Preparations were field stimulated with 5 s trains of pulses, 1 ms, 55 V at 100 s intervals. Responses in the presence of both BW 172*C*58 (10 μM) and atropine (10 μM) are indicated by squares (□), in the presence of BW 172*C*58 (10 μM) alone by triangles (△), and in the absence of both antagonists by circles (○). Open (panel a) and closed (panel b) symbols show responses in the absence and presence of suramin (100 μM), respectively. The vertical axes show peak responses to field stimulation expressed as percentages of the response to noradrenaline (100 μM). The horizontal axes show the frequency (Hz) of field-stimulation. In all cases the points represent the mean ± S.E.M. of 5–6 observations.

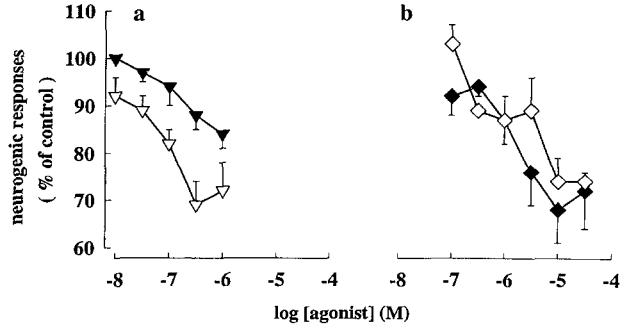

Fig. 6. The effects of suramin (100 μM) on the inhibition of responses to field stimulation by (a) α,β-methyleneATP and (b) adenosine tetraphosphate. The filled symbols indicate agonist effects in the presence of suramin. The vertical axis shows responses to field stimulation in the presence of agonist expressed as a percentage of pre-agonist responses to field stimulation. The horizontal axes show the log molar concentration of agonist. In all cases the points represent the mean ± S.E.M. of 5–6 observations.

4. Discussion

The findings of the present study demonstrate that the naturally occurring nucleotides, UTP, ATP and adenosine tetraphosphate enhance contractile activity of the longitudinal muscle layer of guinea-pig isolated portal vein preparations. Since the effects of ATP were not blocked by 8-phenyltheophylline, and since adenosine was ineffective, the contractile responses to the nucleotides are unlikely to be mediated by a P_1 purinoceptor.

The finding that a number of stable analogues of ATP mimicked its ability to enhance contractile activity is consistent with the possibility that a P_2 purinoceptor is involved. The rank order of potency of the agonists was: 2-methylthioATP > α,β-methyleneATP > adenosine tetraphosphate $\geq$ β,γ-methyleneATP $\geq$ ATP = UTP $\gg$ adenosine. This rank order of potency of the purine nucleotides is not entirely typical of either generally reported for P_{2x} or P_{2y} purinoceptors, i.e.: α,β-methyleneATP > β,γ-methyleneATP > ATP > 2-methylthioATP, and 2-methylthio-ATP $\gg$ ATP > α,β-methyleneATP = β,γ-methyleneATP, respectively (Fredholm et al., 1994).

While we initially considered that the receptor subtype mediating vasoconstriction might be of the P_{2y} subtype, very recently it has been proposed (Kennedy and Leff, 1995; Khakh et al., 1995) that the true order of agonist potency at the P_{2x} purinoceptor is not as outlined by Fredholm et al. (1994) but rather: 2-methylthioATP $\geq$ ATP $\geq$ α,β-methyleneATP = β,γ,-methyleneATP. These authors propose that this rank order is not typically seen because of the susceptibility of ATP and 2-methylthioATP to degradation by ectonucleotidases (Hourani and Chown, 1989; Cusack, 1993). This would distort the rank order of agonist potencies. It may be, therefore, that P_{2x} rather than P_{2y} purinoceptors mediate the contractile effects of some of the agonists, for example, α,β-methyleneATP, used in guinea-pig portal vein. This seems feasible since Bo and Burnstock (1993) have shown, in autoradiographic studies, that P_{2x} purinoceptors are present in the longitudinal muscle layer of this tissue. Moreover, in general, stimulation of P_{2x} purinoceptors in blood vessels leads to vasoconstriction rather than vasodilatation (Pearson and Gordon, 1989).

The finding that suramin blocked the contractile responses elicited by both 2-methylthioATP and $\alpha\beta$-methyleneATP is compatible with the presence of either P_{2x} or P_{2y} purinoceptors, since previous studies indicate it lacks selectivity for either of these receptor subtypes (Dunn and Blakeley, 1988; Hoyle et al., 1990; Leff et al., 1990). This agent did not, however, block responses elicited by either ATP or by adenosine tetraphosphate. While this insensitivity might be explained in part by the ability of suramin to inhibit ectonucleotidases (Hourani and Chown, 1989), the tissue may, in addition to postjunctional P_{2x} and P_{2y} purinoceptors, contain postjunctional 'nucleotide', or P_{2u} purinoceptors (O'Connor et al., 1991; Fredholm et al., 1994). These receptors are known to mediate vasoconstriction in other tissues. An alternative possibility is that ATP and adenosine tetraphosphate may elicit responses by activating a suramin-insensitive P_2 purinoceptor (Von Kügelgen et al., 1990, Von Kügelgen and Starke, 1990) or a pyrimidine receptor (Von Kügelgen and Starke, 1990).

A subset of experiments were undertaken in which the effects of a noradrenergic neurone blocking drug and of atropine, in the absence and presence of suramin, on the responses to field stimulation of intrinsic nerve terminals within the portal vein were investigated. The use of the short acting, but highly selective, noradrenergic neurone blocking drug, BW 172*C*58 (Boura et al., 1960), confirmed that field stimulation of intrinsic nerve terminals activates sympathetic nerves in this tissue, while experiments in which atropine was also present indicated that acetylcholine is unlikely to contribute to the response to field stimulation. Suramin was without effect on field-stimulation-induced responses, prima facie indicating either that the purinergic contribution to excitatory neurotransmission is small, or that the receptors upon which any released ATP might act are suramin-insensitive. The effects of suramin, however, may be confounded by its action to inhibit ectonuceotidases (Hourani and Chown, 1989). Further experiments with pyridoxalphosphate-6-azophenyl-2′-4′-disulfonic acid (PPADS; Lambrecht et al., 1992), and reactive blue 2 (Burnstock and Warland, 1987) are indicated to establish or exclude any contribution of ATP to sympathetic co-transmission to this tissue. In addition, the effects of repeated exposure of preparations to α,β-methyleneATP upon neurotransmission require further investigation.

β,γ-methyleneATP, 2-methylthioATP and UTP produced some inhibition of responses to field stimulation but neither ATP nor adenosine did so. The maximal extent of the inhibition was, however, very small. The rank order of potency found, namely α,β-methyleneATP $\gg$ β,γ-methyleneATP > 2-methylthioATP, is consistent with the possibility that P_{2x} purinoceptors can mediate inhibition of neurotransmission in this tissue. It is not, however, possible to be certain of the location of these receptors, ie. whether it is due to desensitization of post junctional receptors or to a prejunctional action.

Our finding that the endogenous purines, ATP and adenosine, were ineffective in inhibiting neurotransmission is consistent with that of Burnstock et al. (1984), who suggested that inhibition of noradrenergic transmission to the guinea-pig portal vein by purines was unlikely to be of physiological significance. Support for P_2 purinoceptor involvement in mediating inhibitory responses to the stable analogues of ATP comes from the finding that suramin blocked the inhibitory response to α,β-methyleneATP. Suramin was, however, ineffective in modifying the inhibitory responses to adenosine tetraphosphate. A possible explanation is that the receptor activated by adenosine tetraphosphate in this tissue differs from that activated by α,β-methyleneATP. Since repeated application of α,β-

methyleneATP leads to loss of its inhibitory effect on field stimulation, the latter receptor may of the P_{2x} subtype. Alternatively, any antagonism by suramin of adenosine tetraphosphate may be balanced by its action to inhibit the extracellular breakdown of the endogenous nucleotide as discussed above. We have recently observed a similar pattern of suramin sensitivity and insensitivity to these two agonists using preparations of guinea-pig uterine vasculature (Sikorski et al., 1994).

In conclusion, the use of potent and selective ATP analogues in this study indicates the presence of P_2 purinoceptors mediating contractions of the longitudinally-arranged muscle of the guinea-pig portal vein. The P_{2x} purinoceptor subtype may be involved in mediating the contractile effects of some of these agonists, but resistance of the actions of ATP and adenosine tetraphosphate to suramin, and of adenosine tetraphosphate and UTP to desensitization of P_{2x} purinoceptors by α,β-methylene ATP, may indicate the contribution of additional receptor subtype/s. The inhibition of neurogenic contractions by α,β-methyleneATP, together with the ready reversibility of the inhibition seen, and the susceptibility of the response to suramin, indicate that inhibitory P_2 purinoceptors may also be present in the guinea-pig isolated portal vein. The inhibitory action of adenosine tetraphosphate, however, like its excitatory action, was suramin-insensitive. Both adenosine tetraphosphate and ATP can be formed by the extracellular breakdown of diadenosine polyphosphates (Hoyle, 1990; Rodriguez-Pascual et al., 1992). These are present in neurones, chromaffin cells, and possibly platelets, and may play a physiological role in the regulation of contractility in this tissue. Future experiments to characterize the receptors at which these endogenous purine nucleotides act in the regulation of portal vein function is of some importance, since these substances are thought to play a role in regulating liver blood supply (Lautt, 1985; Lee and Filkins, 1988).

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