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Potentiation of adenosine triphospate-induced contractile responses of the guinea-pig isolated vas deferens by adenosine monophosphate and adenosine 5′-monophosphorothioate

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1 The effects of incubating the guinea-pig isolated vas deferens in the presence of adenine nucleotides (adenosine triphosphate, ATP; adenosine diphosphate, ADP; and adenosine monophosphate, AMP), or in the presence of their phosphorothioate analogues (adenosine 5′-O-(3-thiotriphosphate), ATPγS; adenosine 5′-O-(2-thiodiphosphate), ADPβS; and adenosine 5′-monophosphorothioate, AMPαS), on contractile responses to ATP were compared.

2 After challenge with a low (1 μM) or high (300 μM) concentration of ATP to obtain control responses, one vas deferens of a pair was incubated for 5 min with one of the adenine nucleotides, while the contralateral preparation was incubated with the corresponding phosphorothioate analogue. At the conclusion of the incubation the preparations were challenged again with ATP.

3 Incubation with AMP or AMPαS resulted in a transient potentiation of responses to 1 μM and 300 μM ATP. The potentiation following incubation with AMPαS was larger than that produced by AMP.

4 After incubation with ADP, ADPβS, ATP and ATPγS, responses to 1 μM ATP were decreased, while those to 300 μM ATP were unaffected.

5 Thus, incubation with AMP and AMPαS results in potentiation, rather than inhibition, of ATP-induced responses. On the other hand, 5′-diphosphate, 5′-triphosphate, 5′-O-(2-thiodiphosphate) and 5′-O-(3-thiotriphosphate) moieties on adenosine have no effect or cause autoinhibition.

6 These results indicate that AMP exerts a potentiating effect on reactivity to exogenous ATP. AMP arising from the enzymatic degradation of ATP might modulate the level of response to ATP released endogenously as a cotransmitter.

Introduction

Adenosine triphosphate (ATP) causes contraction of a large number of isolated smooth muscle preparations, such as the urinary bladder, vas deferens, nictitating membrane, and certain blood vessels (Burnstock, 1985). If the ATP is allowed to remain in contact with the tissue, it is commonly observed that the response to subsequently administered ATP is diminished (Hogaboom *et al.*, 1980; Fedan *et al.*, 1981; see also Burnstock & Kennedy (1985) for review). This phenomenon has been referred to as desensitization or autoinhibition. The autoinhibition is characteristically specific for nucleotide-induced responses, in that responses to other agonists, such as noradrenaline, are unaffected. Although the mechanism(s) underlying autoinhibition have not been defined, it is known to be caused by other purine nucleotides containing 5′-polyphosphate chains, e.g. adenosine diphosphate (ADP), guanosine triphosphate (Lukacsko & Krell, 1981; 1982); by purine nucleotides containing 5′-anhydride-oxygen substitutions, e.g. adenosine 5′-(α,β-methylene)-triphosphate (Burnstock & Kennedy, 1985); and by pyrimidine nucleotides, e.g. cytosine triphosphate (Lukacsko & Krell, 1981; 1982). Moreover, autoinhibition is usually reversible after removal of the nucleotide. Because it results in a selective antagonism of P_2- purinoceptor-mediated responses, autoinhibition has been used as a means of investigating the role of ATP released as a cotransmitter from sympathetic nerves (Fedan *et al.*, 1981; Burnstock & Kennedy, 1985; Burnstock, 1986).

In contrast to the autoinhibitory effects of adenine nucleotides, contractile responses to noradrenaline of the guinea-pig vas deferens (Holck & Marks, 1978) and guinea-pig and rat portal vein (Kennedy & Burnstock, 1985) were potentiated in the presence of adenine nucleosides and nucleotides (Stone, 1983) by undefined postjunctional mechanisms.

In the course of experiments comparing adenine nucleotides for activity in the guinea-pig vas deferens (Fedan *et al.*, 1986), some tissues were exposed to ATP, at the end of concentration-response comparisons, to assess possible alterations in tissue reactivity resulting from exposure to adenine nucleotides. It was noted that vasa deferentia which had been exposed to adenosine 5'-monophosphorothioate (AMPαS) yielded responses to ATP which were larger than those obtained from contralateral tissues exposed to adenosine monophosphate (AMP). As the AMP and AMPαS had been removed from the baths, this implied that AMPαS induced a long-lasting potentiating effect. In view of the fact that only autoinhibitory interactions between adenine nucleotides have been studied previously, it was decided to examine in more detail the effects of exposure of the vas deferens to adenine nucleotides on its subsquent reactivity. Since the original observation involved AMPαS, suggesting that the effect might be attributable to the phosphorothioate substitution, the studies were extended to include ADP and ATP, and their phosphorothioate-substituted analogues, adenosine 5'-O-(2-thiodiphosphate) (ADPβS) and adenosine 5'-O-(3-thiotriphosphate) (ATPγS).

Methods

Tissue preparation

English short-haired guinea-pigs (350–500 g; Camm Research Institute, Wayne, New Jersey) were killed by a blow to the head and bled. The vasa deferentia were removed and placed in modified Krebs-Henseleit (MKH) solution (composition, mM: NaCl 113.0, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 5.5), cleaned, tied to holders and placed in organ baths (3 ml; 37°C) containing MKH which was gassed with 95% O_2 and 5% CO_2. The tissues were attached to force-displacement transducers for the measurement of isometric contractile responses. An optimum resting load of 0.2–0.4 g was applied. The tissues were equilibrated for one hour, during which time they were washed with fresh MKH at 15 min intervals.

Experimental protocols

The tissues were exposed three times to ATP, either 1 μM or 300 μM, to obtain control responses. The rationale for choosing these concentrations was two fold. Firstly, responses to the low concentration would be the most sensitive to an autoinhibitory effect. Secondly, these concentrations are within the effective concentration-range of ATP in the tissue (Fedan *et al.*, 1986). Both vasa deferentia of a pair were exposed to the same concentration, and only one concentration of ATP was used for a given pair of tissues. In each exposure period, the ATP was present for 3 min. The tissues were then washed with fresh MKH twice at 5 min intervals before the next addition of ATP. Thus, there was 13 min between each ATP addition.

After the control responses were obtained, one tissue of a pair was exposed to AMP, ADP or ATP, while AMPαS, ADPβS or ATPγS, respectively, were added to the contralateral preparations. Submaximally effective concentrations of these nucleotides (Fedan *et al.*, 1982; 1986) were used. After a 5 min incubation the tissues were washed with MKH twice, at 5 min intervals, to remove the nucleotides. The preparations were then exposed three times, at 13 min intervals, to ATP in the same concentrations used to obtain control responses. Thus, 10 min elapsed between washout of the incubated nucleotides and the second series of ATP-induced responses, referred to in the remaining text as 'post-nucleotide' responses.

At the end of the experiment all vasa deferentia were weighed for normalization of contractile responses (g tension) in terms of grams wet tissue weight, i.e. g tension g^{-1} wet weight.

Drugs

ATP (sodium), ADP (sodium), AMP (sodium), ADPβS (lithium) and AMPαS (lithium) were from Sigma Chemical Co., St Louis, Missouri. ATPγS (lithium) was from P-L Biochemicals, Inc., Milwaukee, Wisconsin. Nucleotides were dissolved in saline for use.

Analysis of data

Where appropriate, the results are presented as means ± s.e.mean. *n* is the number of separate experiments. The data were evaluated for differences by use of Student's *t* test for paired samples. The 0.05 level of probability was considered significant.

Results

After a 5 min incubation of vasa deferentia with AMP or AMPαS (10 mM), followed by washout of the agents, the initial responses to both 1 μM and 300 μM ATP were potentiated, when compared with the reference (i.e. third control) response (Figure 1). Only

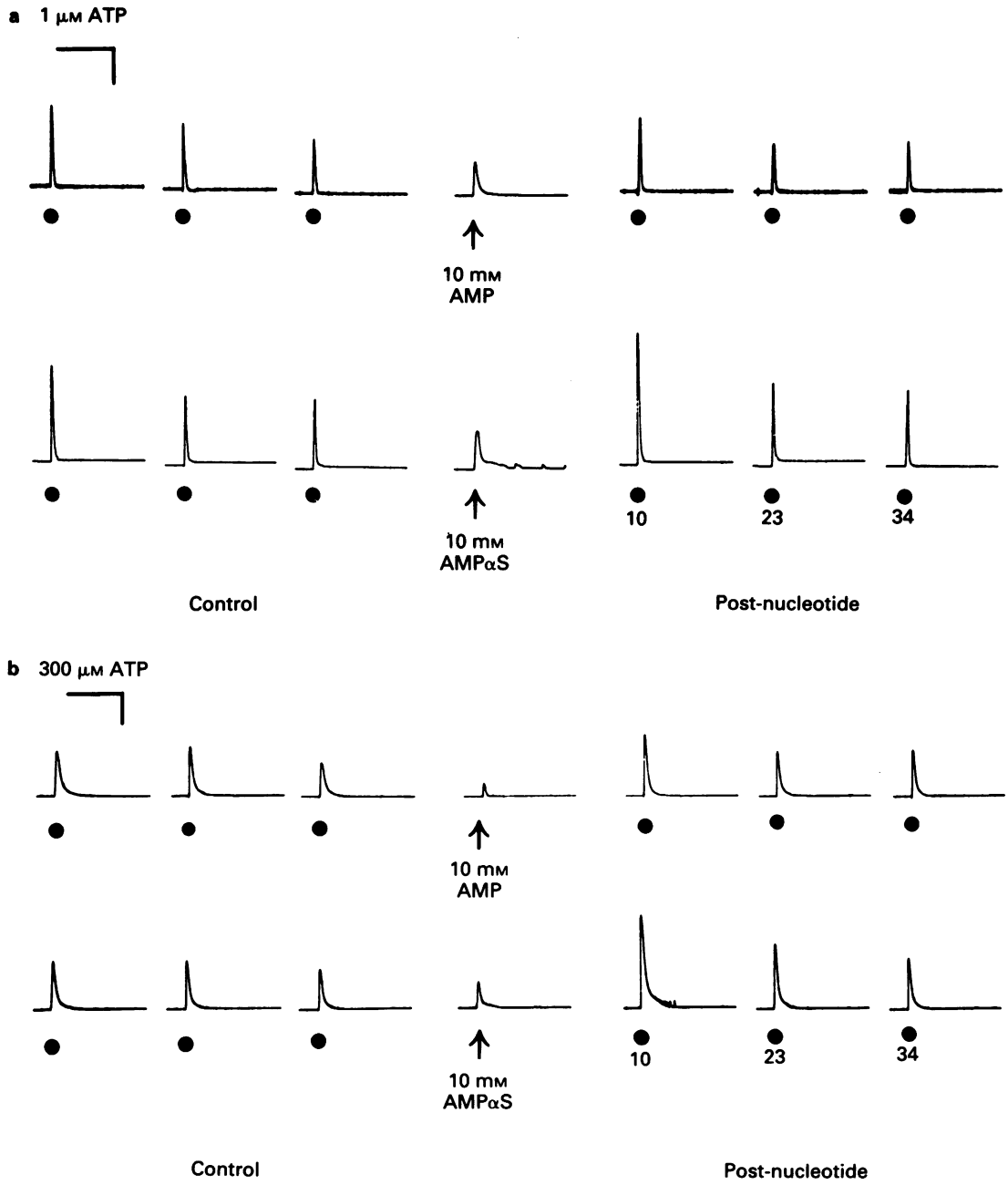

Figure 1 Effect of incubation of the guinea-pig vas deferens with 10 mM AMP or AMPαS on responses to 1 μM (a) or 300 μM (b) ATP. The representative results shown in (a) and (b) were each obtained from paired tissues. Each tissue was exposed to ATP (●) three times at 13 min intervals (3 min exposure to ATP followed by two washes (not shown) before re-addition) to obtain control responses. Tissues were incubated with AMP or AMPαS for 5 min (added at arrows). Following two washes at 5 min intervals (not shown) ATP was added repetitively (●) using a 13 min cycle to obtain post-nucleotide responses. The time (min) elapsed after removing the incubated nucleotide is indicated below the bottom tracing. Horizontal calibration: 1 min; vertical calibration: 1 g (a), 2 g (b).

the first post-nucleotide response, obtained 10 min after washout, was potentiated after incubation with AMP, whereas both the first and second responses, obtained 10 and 23 min after washout of AMPαS, were potentiated. The potentiation seen after AMPαS treatment was significantly larger than that occurring after exposure to AMP. By the third post-nucleotide exposure to both concentrations of ATP, the magnitude of the responses of AMP- and AMPαS-exposed tissues returned to the control level. The results of five such experiments are summarized in Figure 2, wherein the time-course of the decline in the magnitude of potentiation may be seen. Figure 2 (histograms in (a) and (b)) also shows that, in both series of experiments, the contractile responses to AMPαS were significantly larger than those to AMP.

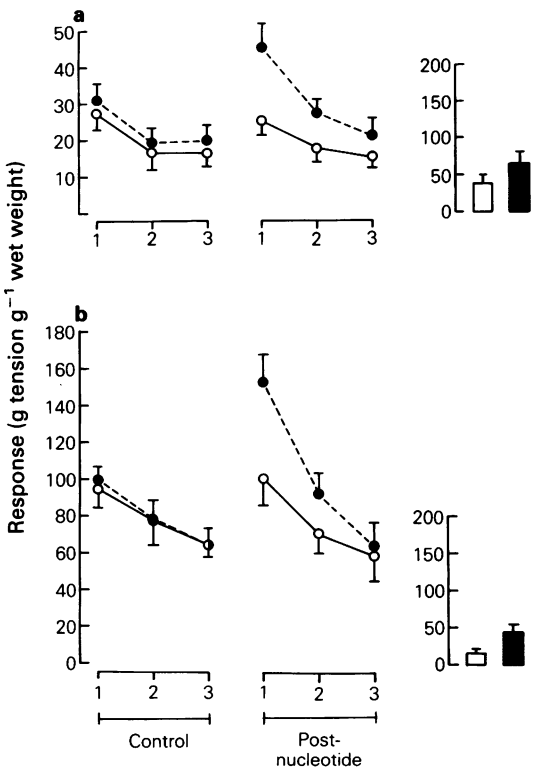

Figure 2 Summary of the effects of incubating the guinea-pig vas deferens with AMP (○) or AMPαS (●) on responses to 1 μM (a) or 300 μM (b) ATP. The 3 control responses are indicated as 1–3, and the post-nucleotide responses 1–3 correspond to those obtained 10, 23, and 36 min after washout of the nucleotides (see Figure 1). The histograms at the right hand side of (a) and (b) indicate the magnitude of the responses to 10 mM AMP (open column) and 10 mM AMPαS (solid column) upon their addition to the organ bath. The responses to AMPαS were significantly larger than those to AMP (columns in (a) and (b)). Each point (column) represents the mean and vertical lines show s.e.mean ($n = 5$).

No responses to 1 μM ATP were obtained after exposure to ATP or ATPγS, during the 36 min post-nucleotide period (Figure 3). In contrast, responses to 300 μM ATP were obtainable after the incubation in ATP or ATPγS (Figure 3), and their magnitude was not different from the control level (Figure 4). The results of five such experiments are summarized in Figure 4. Responses to ATPγS were significantly larger than responses to ATP when these agents were added to begin the incubation (Figure 4, histograms in (a) and (b)).

Incubating tissues with ADP and ADPβS led to effects which were essentially identical to those seen with ATP and ATPγS, with the exception that small responses to 1 μM ATP were obtainable by the third post-nucleotide challenge (Figure 5).

Discussion

Responses to both concentrations of ATP were potentiated after incubation of the tissues with AMP and AMPαS, i.e., neither nucleotide induced autoinhibition. In contrast, ADP, ADPβS, ATP and ATPγS inhibited responses to the low concentration of ATP and had no effect on responses to the high concentration. That is, under no condition did incubation with these latter four compounds lead to potentiation. Thus, the qualitative alteration in responsiveness to ATP would appear to be related to the number of 5′-phosphate moieties irrespective of whether a phosphorothioate substitution is present.

The preliminary observation (see Introduction) that exposure of the vas deferens to AMPαS, compared to incubation in AMP, resulted in potentiation of ATP-induced responses suggested initially that potentiation might be due to the phosphorothioate moiety. Consequently, the phosphorothioate analogues of ADP and ATP were studied to determine if a relationship would evolve between observed potentation and the presence of phosophorothioate. This, however, was not the case. What did become evident in the present study was that incubation with AMP also resulted in potentiation of ATP-induced responses.

The greater potentiating effect of AMPαS compared to AMP might have resulted because the initial contractile responses to AMPαS were larger. However, there appears to be no relationship between the magnitude of the initial contraction *per se* and the onset of potentiation, as ADP, ATP and their phosphorothioate analogues evoked larger responses than AMP or AMPαS, but after washout potentiation was not seen.

It is possible that adenine nucleotides are potentially

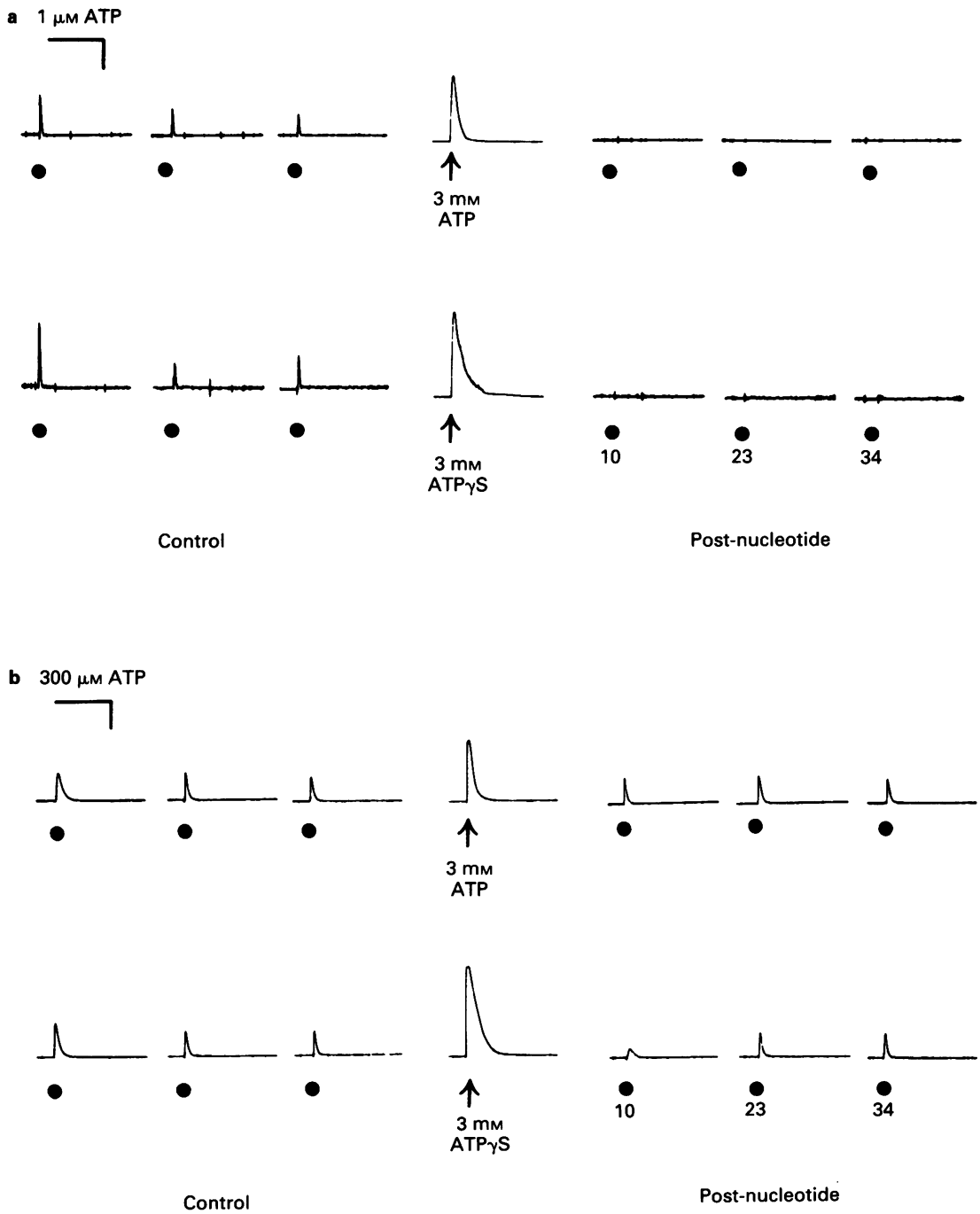

Figure 3 Effect of incubation of the guinea-pig vas deferens with ATP or ATPγS on responses to 1 μM (a) or 300 μM (b) ATP. The representative results shown in (a) and (b) were each obtained from paired tissues. See the legend to Figure 1 for explanation of the protocol, symbols and other details. Horizontal calibration: 1 min; vertical calibration: 1 g (a), 2 g (b).

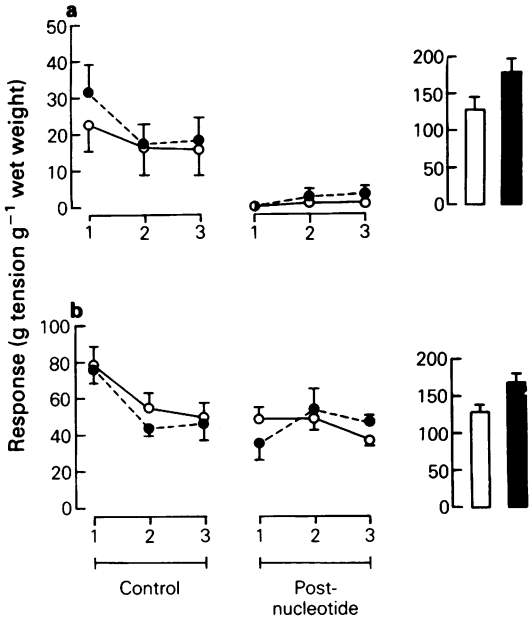

Figure 4 Summary of the effects of incubating the guinea-pig vas deferens with 3 mM ATP (○) or ATPγS (●) on responses to 1 μM (a) or 300 μM (b) ATP. The histograms at the right hand side of (a) and (b) indicate the magnitude of the responses to 3 mM ATP (open columns) and 3 mM ATPγS (solid columns) upon their addition to the organ bath. See Figure 2 legend for details. The responses to ATPγS were significantly larger than those to ATP (histograms in (a) and (b)). Each point (column) represents the mean and vertical lines show s.e.mean ($n = 5$).

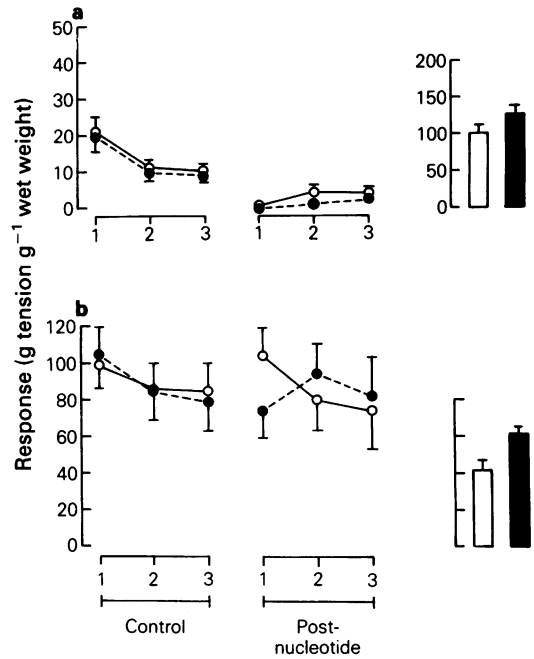

Figure 5 Summary of the effects of incubating the guinea-pig vas deferens with 3 mM ADP (○) or ADPβS (●) on responses to 1 μM (a) or 300 μM (b) ATP. The histograms at the right hand side of (a) and (b) indicate the magnitude of the responses to 3 mM ADP (open columns) and 3 mM ADPβS (solid columns) upon their addition to the organ bath. See Figure 2 legend for details. The responses to ADPβS were significantly larger than those to ADP (columns in (a) and (b)). Each point (column) represents the mean and vertical lines show s.e.mean ($n = 5$).

capable of inducing both autoinhibition and potentiation, and that the resultant effect reflects the balance of the two, opposing actions. The development of autoinhibition could be related to the magnitude of the initial response. Lukacsko & Krell (1982) observed in guinea-pig urinary bladder that, with the exception of AMP and adenosine which did not possess contractile activity, the development of autoinhibition was related to the ability of nucleotides to cause a response. AMP and AMPαS, having little contractile activity compared to ADP, ADPβS, ATP and ATPγS, would be less likely to desensitize the tissues to ATP, thereby allowing the potentiating effect to predominate.

Whether or not autoinhibition resulted after incubation with the nucleotides depended on the concentration of ATP used to evoke the responses. Responses to 1 μM ATP remained depressed up to 36 min after incubation with ATP, ATPγS, ADP and ADPβS, i.e. the effect was not readily reversible. However, the post-incubation responses to 300 μM ATP were not inhibited; in this case the autoinhibition was reversible.

The effect of AMP observed in the present study is somewhat different from that seen in guinea-pig urinary bladder (Lukacsko & Krell, 1982). If it remained present at the time the ATP was added, AMP, while eliciting no response initially, nevertheless inhibited responses to ATP. In the present experiments, AMP had been removed from the bath before the addition of ATP.

The mechanism whereby AMP and AMPαS cause potentiation is unknown. However, the finding that AMPαS produced a greater and longer-lasting potentiation than did AMP is of interest. Pearson *et al.* (1985) observed that ecto-5'-nucleotidase of cultured aortic smooth muscle cells catabolized both AMP and AMPαS, the latter nucleotide having a greater affinity for the enzyme but a lesser V_{max}. Moreover, AMPαS

inhibited competitively the dephosphorylation of AMP. While it is difficult to assess the applicability of these findings to the vas deferens (Pearson *et al.*(1985) found differences between the metabolism of AMPαS by pig aortic smooth muscle and endothelial cells), it is possible that incubation of the vas deferens with AMPαS inhibits ecto-5'-nucleotidase activity. Responses to ATP might be potentiated by the accumulation in the receptor biophase of AMP, which has some, albeit weak, contractile activity. This notion requires that the enzyme would remain inhibited for at least 23 min (see Figure 2). Prolonged inhibition of enzyme activity is conceivable if the active site of 5'-nucleotidase becomes thiophosphorylated in the manner described for certain kinase enzymes when ATPγS is substrate (Gratecos & Fischer, 1974; Sherry *et al.*, 1978). Dethiophosphorylation of the active site of kinase enzymes proceeds more slowly than dephosphorylation, resulting in reduced enzyme turnover rate (V_{max}) and in an apparent inhibition of activity against the biological substrate. Both of these effects were observed by Pearson *et al.* (1985), and they could retard the breakdown of AMP for as long as the enzyme is thiophosphorylated. The difference in the effects of AMP and AMPαS may thus be interpreted in relation to interactions of AMPαS with 5'-nucleotidase, but the mechanism of the potentiating effect of AMP *per se* is presumed to apply to AMPαS.

The present results suggest that AMP could modulate the response to neurally-released ATP, in a manner similar to that described for adenine nucleotides and adenosine affecting noradrenaline-induced responses (see Introduction). However, it is the response to neurally-derived ATP, rather than noradrenaline, which is modulated. Since several minutes is required for offset of the potentiating effect, AMP might be involved in the long-term regulation of reactivity to ATP. While autoinhibition is induced by exogenously applied adenine nucleotides, the degree to which the contractile response to endogenously-released ATP is affected by the onset of autoinhibition is unknown. Perhaps the potentiating effect of AMP is to begin the recovery from autoinhibition caused by endogenous ATP, in order to restore reactivity to ATP to a desirable level.

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