Br. J. Pharmac. (1977), **59**, 163–175

INTERACTIONS BETWEEN THE EFFECTS OF α- AND β-ADRENOCEPTOR AGONISTS AND ADENINE NUCLEOTIDES ON THE MEMBRANE POTENTIAL OF CELLS IN GUINEA-PIG LIVER SLICES

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1 The β-adrenoceptor agonist isoprenaline normally causes only a small and inconsistent increase in the membrane potential of cells in guinea-pig liver slices, in contrast to the large hyperpolarizations seen with α-agonists. However, after a selective α-adrenoceptor agonist has been applied, the response to isoprenaline becomes greatly enhanced.

2 Simultaneous application of small doses of an α- and a β-agonist produce hyperpolarizations larger than the sum of the responses to each agent alone.

3 These interactions occur with a range of sympathomimetic amines, including some which are not substrates for various processes for the uptake and inactivation of catecholamines.

4 Hyperpolarizations caused by externally applied cyclic adenosine-3′,5′-monophosphate (cyclic AMP) also become larger after application of an α-agonist.

5 The adenine nucleotides adenosine 5′-diphosphate (ADP) and adenosine 5′-triphosphate (ATP) hyperpolarize guinea-pig liver cells in the dose range 0.1–1.0 mM. This response is not increased after an α-agonist. However, ADP and ATP are themselves able to enhance the response to β-agonists.

6 These interactions between α-agonists, β-agonists and adenine nucleotides seem to involve steps subsequent to receptor activation. Changes in the intracellular actions of cyclic AMP may be concerned.

Introduction

In addition to their well-established effects on glucose release, adrenaline and noradrenaline *in vivo* cause a net loss of potassium from the livers of several species. Both actions can be demonstrated *in vitro* using liver slices which thus provide a convenient preparation for the study of the underlying mechanisms. Experiments with guinea-pig liver slices have shown that the potassium loss is accompanied by an increase in membrane potential and a fall in membrane resistance, and can be explained as a consequence of an increase in the potassium permeability of liver cell membranes (Haylett & Jenkinson, 1972a). This effect on membrane permeability appears to be mediated by α-adrenoceptors, as is that in intestinal smooth muscle (Jenkinson & Morton, 1967; Bülbring & Tomita, 1969), whereas glucose release from guinea-pig and rabbit liver slices is increased by activation of either α- or β-adrenoceptors (Haylett & Jenkinson, 1972b; Haylett, 1976).

Attempts to classify the receptors more exactly revealed a curious phenomenon. If α-receptors alone were to control the effect on potassium permeability, isoprenaline should increase potassium loss and membrane potential only at high concentrations, if at all. While this was usually the case, a few preparations were unexpectedly responsive to isoprenaline. Further, a chance observation showed that even unresponsive preparations became sensitive to isoprenaline if it was applied up to 20 min after the tissue had been exposed to the selective α-receptor agonist, amidephrine. The aim of the present work was to examine the pharmacological characteristics of this phenomenon in the hope of finding out more about the mechanism of action of catecholamine receptors. Preliminary accounts of some of the results have already been published (Haylett & Jenkinson, 1973; Koller, 1976).

Methods

All experiments were carried out at 38°C with liver slices prepared from male guinea-pigs of the Porton or Hartley strains. Since the liver is enclosed in a capsule of connective tissue, the first slice from the surface was discarded. Care was also taken to avoid the region containing large blood vessels in the centre of the lobe.

Up to 8 slices (250–350 μm thick) were prepared for each experiment and transferred immediately to a 50 ml conical flask containing 25 ml of the basic incubation fluid. This had the composition (mM): NaCl 125, KCl 6, $CaCl_2$ 1, $MgSO_4$ 1.2, NaH_2PO_4 1, $NaHCO_3$ 15, Na pyruvate 2, and was bubbled with a 95% O_2 and 5% CO_2 gas mixture. The flask and its contents were then placed in a bath at 38°C and shaken at 120–140 strokes/minute. After 30 min the slices were transferred to fresh incubation fluid, and again after a further 60 minutes. After a total incubation of 3 h a single slice was taken and pinned with stainless-steel needles to a convex Perspex platform. This was mounted in a water-jacketed Perspex bath (total volume 1 ml) through which a continuous stream of pre-warmed incubation fluid was fed by gravity from a reservoir held some 60–70 cm above the bath. Other reservoirs contained the various drug solutions to be tested and could be connected to the bath by a two-way tap. The flow-rate was maintained at 5–10 ml/min throughout (but for the brief interruption when the tap was turned) so that changes in the composition of the fluid bathing the slice were largely complete within 45 seconds.

In some experiments, as indicated in the text, the normal fluid was replaced by a chloride-free variant containing (mM): Na isethionate 125, K methylsulphate 6, $CaSO_4$ 1.5, $MgSO_4$ 1.2, NaH_2PO_4 1, $NaHCO_3$ 15, and sodium pyruvate 2. This was also gassed with 5% CO_2 in O_2.

Further details of the preparation and incubation of the slices, and of their ionic compositions in both normal and chloride-free fluids, can be found in an earlier paper (Haylett & Jenkinson, 1972a).

Electrical recording

Membrane potentials were measured in the conventional way with glass microelectrodes filled with potassium chloride (3 M, acidified to pH 2.5–4). Electrode resistances ranged from 10 to 35 MΩ. The potentials were displayed on an oscilloscope (Tektronix 502A) and on a heated-stylus chart recorder (Devices M2). The tracings shown are from the latter. As in earlier work (Haylett & Jenkinson, 1972a), the recordings were generally made from the second or third cell encountered on lowering the microelectrode into the slice. It was often possible to 'hold' the same cell for over 30 min and on occasion for as long as 90 minutes. All the results to be described are based on comparisons of the effects of drugs on continuously-recorded membrane potentials. This was thought preferable to a technique based on sampling from different cells, partly because the time course of the responses to drugs could be observed more easily, and also in view of the known heterogeneity of liver cells. It is likely that most, if not all, of the present recordings were from parenchymal cells since these make up from 85 to 95% of the cell mass of the liver (see discussion by Haylett & Jenkinson, 1972a). This is also consistent with the demonstration (Green, Dale & Haylett, 1972) that isolated parenchymal cells maintained under short-term tissue culture conditions have the same resting potential (−30 to −40 mV) as that observed in the present work, and show similar changes in membrane potential in response to the application of sympathomimetic amines.

Materials and drugs

Inorganic salts of Analar quality were used in the preparation of bathing solutions, and were made up in glass-distilled water which had been passed through a column of mixed-bed ion exchange resin (Zerolit DM-F). Sodium isethionate was obtained from Koch-Light, potassium methylsulphate from Hopkins & Williams, and sodium pyruvate from Sigma.

Drugs used were (−)-noradrenaline bitartrate and (−)-phenylephrine hydrochloride (Koch-Light); adenosine; adenosine 5′-monophosphoric acid (AMP, sodium salt); adenosine 3′,5′-cyclic-monophosphoric acid (cyclic AMP, sodium salt); adenosine 5′-diphosphate (ADP, sodium salt); adenosine 5′-triphosphate (ATP, sodium salt); naphazoline hydrochloride; (±)-propranolol hydrochloride and papaverine (Sigma); theophylline hydrate (BDH); phentolamine mesylate and xylometazoline hydrochloride (Ciba).

The following were gifts, (±)-amidephrine mesylate and (−)-amidephrine hydrochloride (Mead Johnson); (−)-isoprenaline bitartrate (Ward & Blenkinsop); oxymetazoline hydrochloride and salbutamol sulphate (Allen & Hanbury); methoxamine hydrochloride (Burroughs Wellcome); tetrahydrozoline hydrochloride (Pfizer); tramazoline hydrochloride (Boehringer Ingelheim); thymoxamine hydrochloride (William R. Warner) and ICI 63-197 (2-amino-6-methyl-5-oxo-4-*n*-propyl-4,5-dihydro-*s*-triazololo (1,5-a) pyrimidine).

Stock solutions (1–10 mM) of drugs were made up in distilled water just before each experiment began. Final dilutions were made a few minutes before use. Cyclic AMP, adenosine, AMP, ADP and ATP were dissolved in the appropriate volume of bathing fluid, again just before use.

Results

In contrast to α-adrenoceptor agonists, isoprenaline generally causes only a small and inconsistent increase in the membrane potential of cells in guinea-pig liver slices (Haylett & Jenkinson, 1972b). However, it was found that following a 1–2 min application of the α-agonist amidephrine, low concentrations (10–50 nM) of isoprenaline produced substantial hyperpolarizations. This increase in responsiveness (which

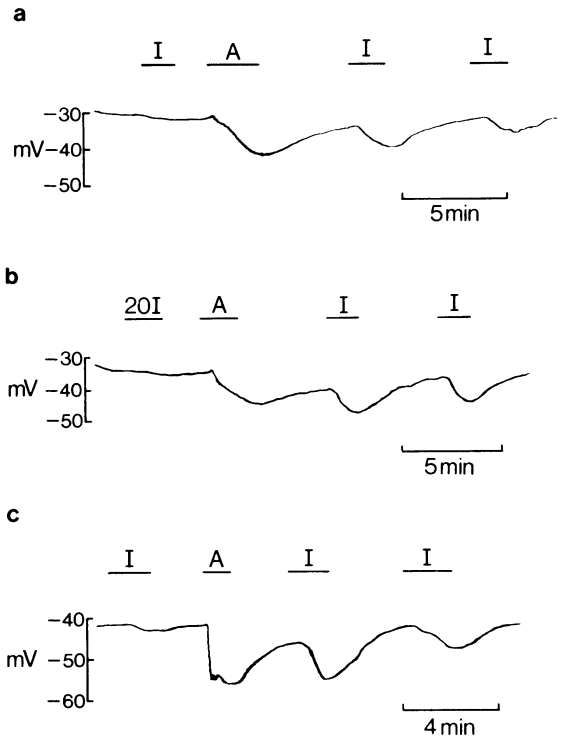

Figure 1 (a) Potentiation by the α-adrenoceptor agonist (−)-amidephrine (10 μM, at A) of the effect of (−)-isoprenaline (50 nM, at I) on the membrane potential recorded from a cell in a guinea-pig liver slice. (b) As in (a) except that the first application of isoprenaline was at a 20-fold higher concentration (1 μM, at 20I). (c) As in (a) but from another preparation bathed in chloride-free (isethionate) solution.

for brevity will be referred to as 'potentiation') is illustrated in Figure 1, and was seen in all but 3 of 83 preparations tested, each from a different guinea-pig. It became particularly striking when the initial response to isoprenaline was negligible. An example of this is shown in Figure 1b; after amidephrine, isoprenaline at 50 nM produced a much greater hyperpolarization than did a 20-fold larger concentration beforehand.

A full analysis of potentiation would require the construction of dose-response curves for isoprenaline applied before, together with, and at various times after a range of concentrations of amidephrine. This has not been attempted in the present work. Our aim was instead to establish the conditions under which potentiation could be seen, and to examine some of its pharmacological characteristics, with a view to understanding the underlying mechanism.

As shown in Figure 1c, the phenomenon still occurred when the chloride content of the bathing fluid was replaced by the larger and presumably less permeant anion isethionate. Since the hyperpolarizations caused by sympathomimetic amines become larger and more rapid in both onset and decline in the absence of chloride (Haylett & Jenkinson, 1972a, b), it was often more convenient to study potentiation under these conditions. As in the earlier work, it was noted that fewer cells failed to respond to isoprenaline before amidephrine when the slices had been equilibrated in chloride-free solution. Nevertheless, potentiation was still easy to observe. Thus before amidephrine (20 μM of the (±)-isomer, or 10 μM of the (−)-isomer), (−)-isoprenaline (50 nM) produced a hyperpolarization of 4.9 ± 0.4 mV (s.e. mean, $n=23$). This rose to 12.8 ± 1.0 mV ($n=23$) 3–5 min after amidephrine.

The aim of the main series of experiments was to test whether potentiation could also be observed with other α- and β-agonists. Cyclic AMP was examined as well, in view of its suggested role in many β-mediated responses. The results have been summarized in Table 1, and will be discussed under the listed headings.

Table 1 Agents that either potentiated the effects of other agonists on the membrane potential of cells in guinea-pig liver slices or whose effects could be potentiated

A			B
Agents able to cause potentiation			*Agents whose effects could be potentiated*
(i)	(ii)	(iii)	
Amidephrine	Noradrenaline	ADP	Isoprenaline
Naphazoline	Phenylephrine	ATP	Salbutamol
Oxymetazoline	Methoxamine		Cyclic AMP
Tramazoline			
Tetrahydrozoline			
Xylometazoline			

See text for explanation of the sub-categories (i), (ii) and (iii).

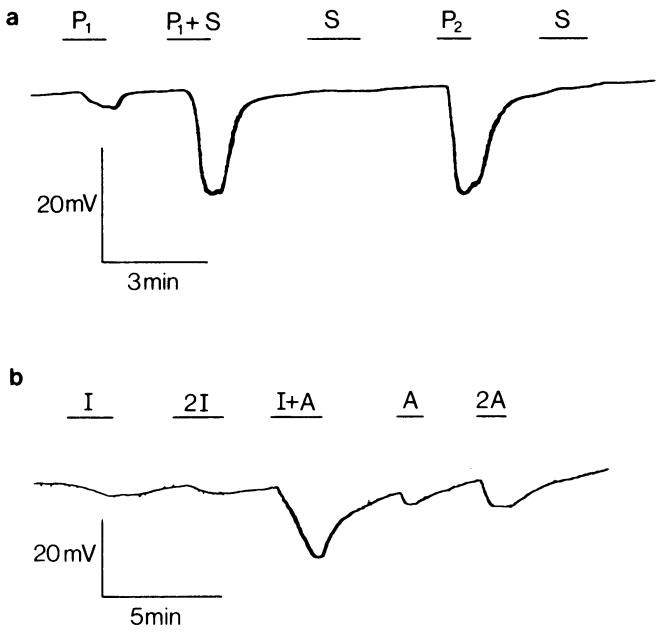

Figure 2 (a) Membrane potential responses to low doses of (−)-phenylephrine (2 μM, at P_1) and salbutamol (0.2 μM, at S) applied separately and in combination (at P_1+S); also shown is the inability of a higher concentration of (−)-phenylephrine (8 μM, at P_2) to potentiate the response to a subsequent application of salbutamol; chloride-free conditions. (b) Responses from another preparation to low doses of (−)-isoprenaline (25 nM, at I) and (−)-amidephrine (2 μM, at A) applied separately and in combination (at I+A). Also illustrated are the responses to twice the concentration of both (−)-isoprenaline (50 nM, at 2I) and (−)-amidephrine (4 μM, at 2A).

Agents that caused potentiation

The sympathomimetic amines listed in columns (i) and (ii) of Table 1 were chosen for their known α-agonist properties (Mujić & van Rossum, 1965; Sanders, Miller & Patil, 1975). All were able to cause both potentiation and hyperpolarization in the dose range 1–50 μM. However, an interesting difference was seen between amidephrine and the various imidazolines on the one hand, and noradrenaline, phenylephrine and methoxamine (column ii) on the other. That is, an increased response to a β-agonist (or to cyclic AMP, see later), could be detected at any time up to 15 to 30 min after a single application of one of the first group of α-agonists, whereas members of the second group caused potentiation only when applied together with the β-agonist, as illustrated for phenylephrine in Figure 2a.

Figure 2b shows a control experiment to demonstrate that the increased response seen on applying an α- and a β-agonist together is not simply a consequence of a non-linear relationship between the concentration of a hyperpolarizing agent and the resulting rise in membrane potential. Thus doubling the concentration of either amidephrine or isoprenaline caused a smaller increase in response than did combining the low doses of each agonist.

The finding that all the α-agonists tested caused both hyperpolarization and potentiation suggested these actions were α-adrenoceptor mediated. In keeping with this, both effects could be reversibly inhibited by phentolamine at 10 μM, as shown in Figure 3. That this relatively high concentration is required agrees with previous results (Haylett & Jenkinson, 1972b) which had shown that phentolamine is much less effective in blocking responses to α-agonists in guinea-pig liver than in other tissues. Thus the dose-ratio with phentolamine at 10 μM was only 13 in the earlier study, as compared with the value of more than 500 to be expected from measurements with a variety of smooth muscles (see e.g. Sheys & Green, 1972). A similar discrepancy with the α-antagonist thymoxamine was seen in the present work. This agent, when tested at 10 μM against the hyperpolarizing action of amidephrine, gave a dose-ratio of 11 in one experiment, and 13 in another, whereas a value of at least 100 would have been expected from studies with smooth muscle from several species, including the guinea-pig (Birmingham & Szolcsányi, 1965). In view of these differences

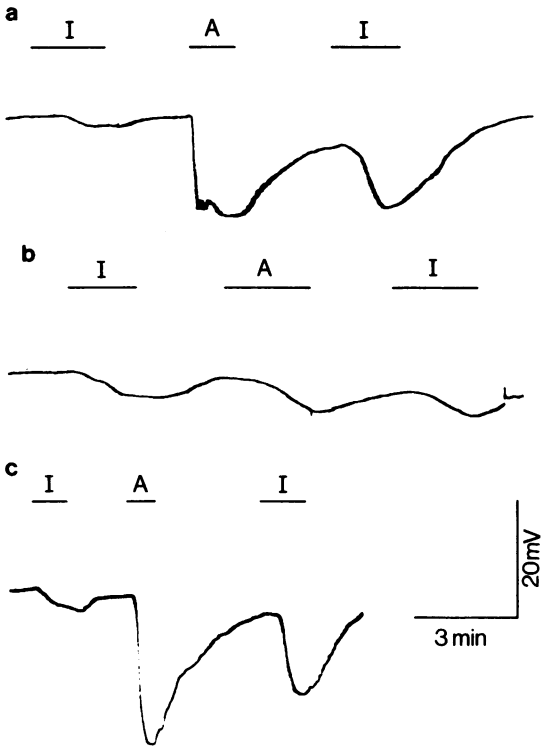

Figure 3 The effect of phentolamine on the potentiation of the response to (−)-isoprenaline (50 nM, at I) by (−)amidephrine (10 μM, at A); chloride-free conditions. (a) Control responses; (b) in the presence of phentolamine (10 μM) applied 3 min before the first dose of isoprenaline; (c) 70 min after washout of the antagonist.

between liver and other tissues it would seem best to continue to describe the receptor concerned in the liver as 'α-like' until its pharmacological characteristics have been studied further.

Agents that could be potentiated; salbutamol and cyclic AMP

Agents that could be potentiated by prior or simultaneous application of α-agonists are listed in Table 1B. As Figure 4 illustrates, the phenomenon was as clearcut with salbutamol as with isoprenaline (see also Figure 10). Since salbutamol is not a substrate for catechol-*O*-methyltransferase, this finding rules out the rather unlikely possibility that the increased response to isoprenaline was a consequence of a transient inhibition of this enzyme by the α-agonist, leading in turn to an increase in isoprenaline concentration in the vicinity of the β-receptors. It seemed more likely that potentiation reflected a change either in the process of β-receptor activation, or in one or more of the steps which follow activation. One of the earliest of these steps in guinea-pig liver, as in many other tissues, is an increase in the intracellular cyclic AMP concentration (see Osborn, 1975). It was interesting for this reason to test whether externally applied cyclic AMP would also hyperpolarize guinea-pig liver cells, as has been demonstrated in rat liver (Friedmann, Somlyo & Somlyo, 1971; Dambach & Friedmann, 1974) and, if so, to determine whether the response became greater after an α-agonist had been applied.

It was found that under the usual experimental conditions, cyclic AMP caused only a small increase in membrane potential; at 1 mM the hyperpolarization was barely detectable (1.2 ± 0.3 mV, $n=9$). As Figure 5a shows, the response became much larger after amidephrine, just as had been observed with β-agonists. Similar results (Figure 5b) were obtained in chloride-free solution though it was noted that cyclic AMP, like isoprenaline, became more effective under these conditions. Thus, before amidephrine, cyclic AMP (0.5 mM) produced a hyperpolarization of 4.5 ± 0.5 mV ($n=10$). However, potentiation was still striking; after amidephrine (10 μM of the (−)-isomer) the same concentration

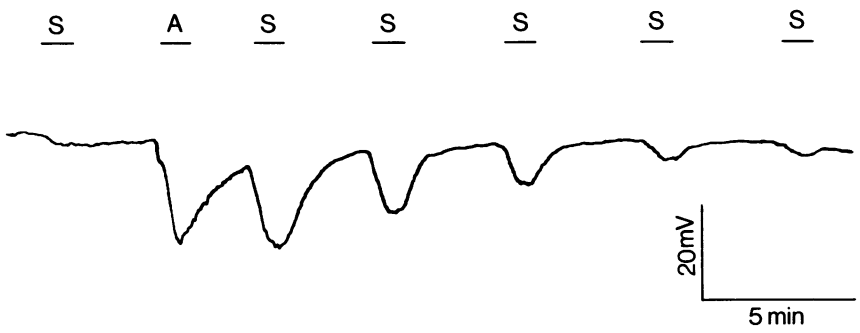

Figure 4 Potentiation by (±)-amidephrine (20 μM, at A) of the effect of salbutamol (0.5 μM, at S) on the membrane potential: chloride-free conditions.

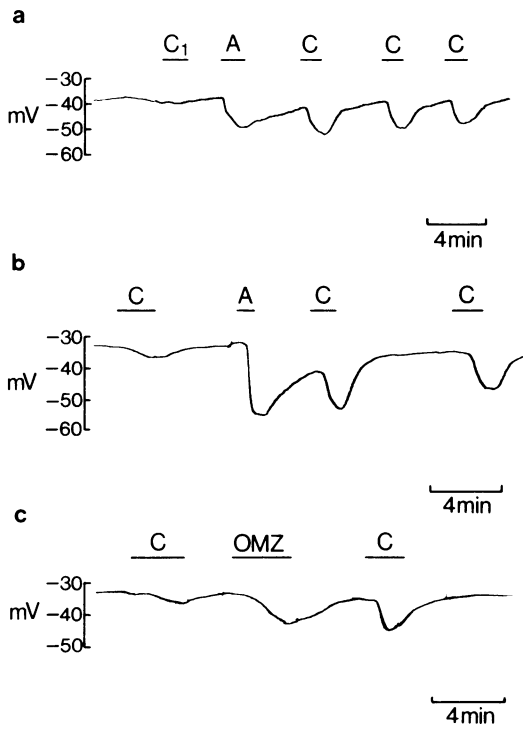

Figure 5 (a) Potentiation of the membrane potential responses to cyclic AMP (2 mM, at C_1: 0.5 mM at C) by (−)-amidephrine (10 μM, at A). (b) From another preparation bathed in chloride-free medium; although responses to cyclic AMP (0.5 mM, at C) are larger under these conditions, potentiation following (−)-amidephrine (10 μM, at A) is still clear-cut. (c) As in (b) but showing potentiation of cyclic AMP by the α-agonist oxymetazoline (2 μM, at OMZ).

of cyclic AMP caused a hyperpolarization of 13.1 ± 0.9 mV ($n=9$). This could as readily be shown with other α-agonists, for example oxymetazoline (see Figure 5c). The response to cyclic AMP before and after amidephrine was unaffected by (±)-propranolol at 1 μM, a concentration sufficient to reduce greatly the effects of isoprenaline (50 nM) on glucose release from guinea-pig liver slices, and on ^{42}K efflux both before and after this α-agonist (Haylett & Jenkinson, 1972b; 1973).

It is commonly assumed that responses to externally applied cyclic AMP reflect an increase in the concentration of the nucleotide within the cell. This assumption is perhaps better founded for the liver than for other tissues, since liver cell membranes are appreciably permeable to cyclic AMP (Exton, Lewis, Ho & Park, 1972; Strange & Percy-Robb, 1975). Accepting that externally applied cyclic AMP does increase the concentration in the cytoplasm, the finding that it produces a greater hyperpolarization after amidephrine is clearly in keeping with the idea that potentiation of the response to a β-agonist occurs at a stage subsequent to both β-adrenoceptor and adenylate cyclase activation.

Effects of other adenine nucleotides

Quite different results were obtained with other adenine nucleotides. ADP and ATP markedly hyperpolarized guinea-pig liver cells, whereas AMP and adenosine were inactive at concentrations up to 1 mM, even after amidephrine. Figure 6a summarizes the effects of these compounds on membrane potential, together with those of cyclic AMP and (−)-amidephrine. ADP and ATP appeared to be about 250 and 160 times less active respectively than (−)-amidephrine, although these potency differences could well be over-estimates. This is because the responses to ADP and ATP were somewhat different in time course from those to amidephrine, being faster in both onset and decline and often 'fading' during the test exposure. These differences were particularly clear-cut in chloride-free solution, as illustrated in Figure 6b. The maximum responses to ADP and ATP may therefore have been underestimated since 'fade' may have been substantial by the time that the agonist had equilibrated with the tissue (see e.g. Paton & Waud, 1964). From Figure 7 it can be seen that amidephrine caused no change in the responses to ADP or ATP at a time when β-mediated responses were substantially potentiated. With this result in mind, it was interesting to test whether these agents could themselves increase the response to a β-agonist. Figure 8 shows that ADP and ATP resembled noradrenaline, methoxamine and phenylephrine in that each caused potentiation, but only when applied together with the β-agonist. However, at doses producing the same hyperpolarization, both nucleotides seemed less effective in this regard than α-agonists. This could reflect the more transient nature of the ADP and ATP responses.

The effects of phosphodiesterase inhibitors

Cyclic AMP is inactivated by the enzyme phosphodiesterase, of which at least two forms exist in the liver, one membrane bound and the other in the cytoplasm (Russell, Terasaki & Appleman, 1973; Appleman & Terasaki, 1975). Thus agents such as papaverine, theophylline and ICI 63-197 (see Davies, 1973) which inhibit phosphodiesterase would in general be expected to increase responses mediated by cyclic AMP in the liver, as elsewhere. In keeping with this, the hyperpolarization caused by salbutamol (100 nM) became greater and more rapid in onset in the presence of papaverine (25 μM). This was seen in four of the five preparations tested; although the dose-response relation for papaverine was not explored in

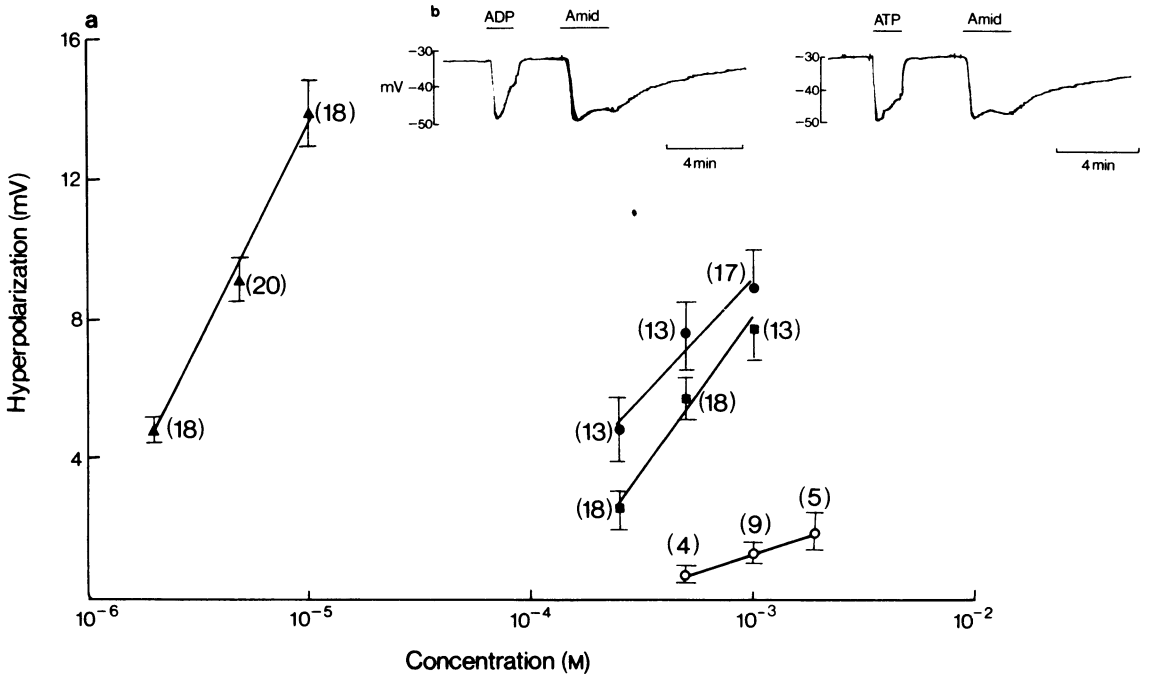

Figure 6 (a) Dose-response relations for the effects on membrane potential of (−)-amidephrine (▲), ATP (●), ADP (■) and cyclic AMP (○). The values plotted are the means, ± s.e. of the number of observations shown in brackets. Normal bathing solution. (b) Comparisons of the hyperpolarizations caused by (−)-amidephrine (5 μM, at Amid), ADP (0.4 mM) and ATP (0.4 mM) in chloride-free medium where 'fading' of the responses to ADP and ATP was particularly evident (see text). Records from the same cell.

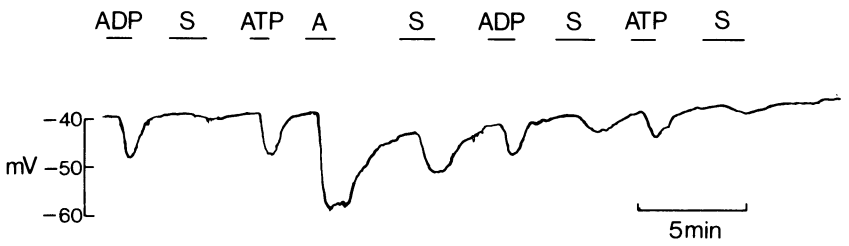

Figure 7 The effects of (−)-amidephrine (10 μM, at A) on responses to salbutamol (0.2 μM, at S) and to low doses of the adenine nucleotides ADP (0.25 mM) and ATP (0.125 mM). The α-agonist potentiates only the responses to the β-agonist. Chloride-free conditions.

any detail, the effect was noticeable at 5 μM and became maximal at about 25 μM. However, as illustrated in Figure 9a, this potentiation was not maintained, and indeed was followed by a period of inhibition even when papaverine had been withdrawn. In two of the four responsive preparations, the recovery was incomplete even after the slice had been washed with papaverine-free solution for more than an hour.

In the presence of papaverine, again at 25 μM, the hyperpolarization caused by low doses of the α-agonist amidephrine (0.5 μM) also sometimes became larger and more rapid in onset (see Figure 9b). Of eight preparations tested in this way, four showed reversible increases in response, two showed increased responses followed by inhibition, and the remainder were unaffected. In a few preliminary experiments theophylline (100–400 μM) and ICI 63-197 (25–50 μM) gave qualitatively similar results to papaverine. That phosphodiesterase inhibition should alter the response to an α-agonist such as amidephrine might seem surprising since, in contrast to

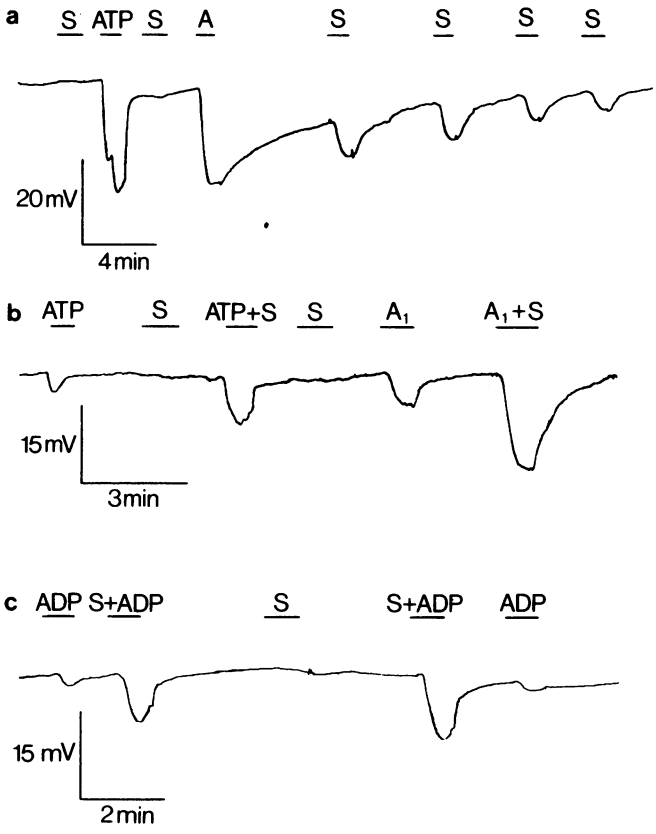

Figure 8 (a) The effects of ATP (0.5 mM) and (−)-amidephrine (10 μM, at A) on the responses to salbutamol (0.1 μM, at S). Whereas (−)-amidephrine is able to potentiate subsequent salbutamol responses, ATP is ineffective in this respect; chloride-free conditions. (b) Responses to low doses of ATP (50 μM) and salbutamol (0.2 μM, at S) applied separately and in combination (at ATP+S). Also shown for comparison are responses to (−)-amidephrine applied alone (4 μM, at A_1) and together with this dose of salbutamol (at A_1 + S); chloride-free conditions. (c) The effects of low doses of ADP (0.1 mM) and salbutamol (0.2 μM, at S) applied separately and in combination (at ADP+S). From the same preparation as (b) and under the same conditions.

isoprenaline, amidephrine does not increase cyclic AMP levels in guinea-pig liver slices (Osborn, 1975). A possible explanation is that adenylate cyclase has a basal activity even in the absence of agonists. If this is so, inhibition of phosphodiesterase should cause cyclic AMP to accumulate, as though a β-agonist had been applied. The increased response to amidephrine (cf. Figure 9) would then be equivalent to that observed when amidephrine is applied together with a β-agonist.

However, the finding that the potentiating action of phosphodiesterase inhibitors often changed to inhibition made it clear that additional factors must have been at work. Whatever the explanation for this effect, its occurrence evidently limits the value of phosphodiesterase inhibitors (certainly those so far examined) as tools for the study of the membrane actions of catecholamines in this tissue.

The role of electrogenic transport

A conceivable explanation for potentiation was that it might be associated in some way with changes in electrogenic transport. This did not seem very probable since earlier work had shown that although electrogenic transport can occur in guinea-pig liver slices, it does not underlie the hyperpolarizations caused by noradrenaline and isoprenaline. The main evidence for the existence of electrogenic transport is that restoration of external potassium to liver slices bathed in a nominally potassium-free solution increases the membrane potential, an effect which is reversibly abolished by ouabain. In contrast, the hyperpolarizations caused by noradrenaline and isoprenaline are insensitive to ouabain at the same concentration, and are better accounted for in terms of an increase in the

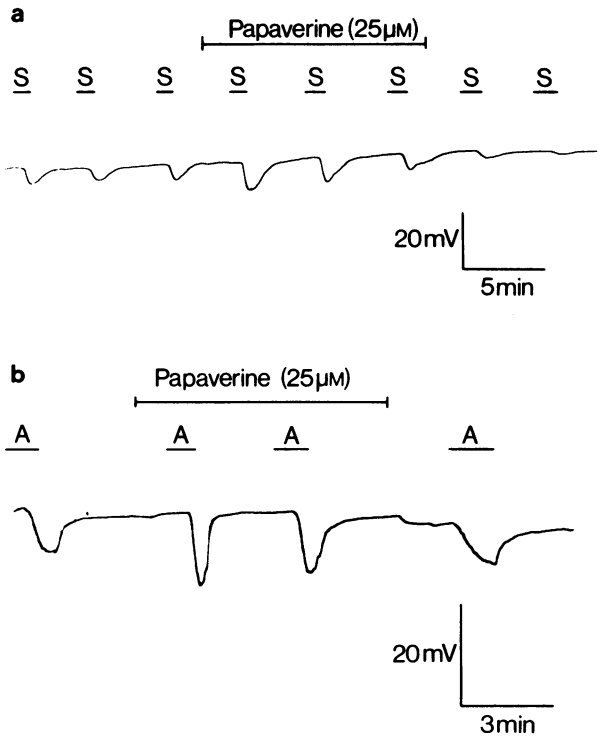

Figure 9 The effects of papaverine (25 μM) on membrane potential responses to (a) salbutamol (0.1 μM, at S) and (b) (±)-amidephrine (5 μM, at A); chloride-free conditions.

potassium permeability of the cell membrane (Haylett & Jenkinson, 1972b; 1973).

Nevertheless it seemed worth testing whether electrogenic transport became more pronounced at a time when the response to a β-agonist had been increased by prior application of an α-agonist. The outcome of such an experiment is illustrated in Figure 10, where it can be seen that the hyperpolarization resulting from potassium restoration was little changed at a time when potentiation was clearly evident.

Thus potentiation does not seem to involve a change in potassium-dependent electrogenic transport. Similarly the fact that potentiation continues in slices bathed in chloride-free solutions rules out mechanisms based on changes in chloride transport.

Discussion

The first of the two main findings is that 'potentiation' in guinea-pig liver is not a specific feature of the agonist pair amidephrine/isoprenaline with which it was initially observed. It has now been shown to occur with a range of sympathomimetic amines, including some which are not substrates for various processes for the uptake and inactivation of catecholamines. This makes it unlikely that changes in drug concentration in the immediate vicinity of the receptors could account for the increase in the response to a β-adrenoceptor agonist applied shortly after an α-agonist. The second main finding, i.e. that the hyperpolarization caused by externally applied cyclic AMP also became larger after an α-agonist, is in keeping with this.

A more puzzling observation was that some agents, e.g. noradrenaline and ATP (see Table 1, columns ii and iii) potentiated the response to isoprenaline and salbutamol only when applied at the same time whereas even a brief exposure to amidephrine or the various imidazolines (column i of Table 1) was followed by an increase in β-responsiveness which lasted for many minutes. This may have been simply a consequence of the rather longer duration of action of the latter α-agonists, as judged by the rate at which the hyperpolarization they elicited subsided after washout. However, potentiation could still be detected even when the membrane potential had returned to its previous level. The possibility remains therefore that

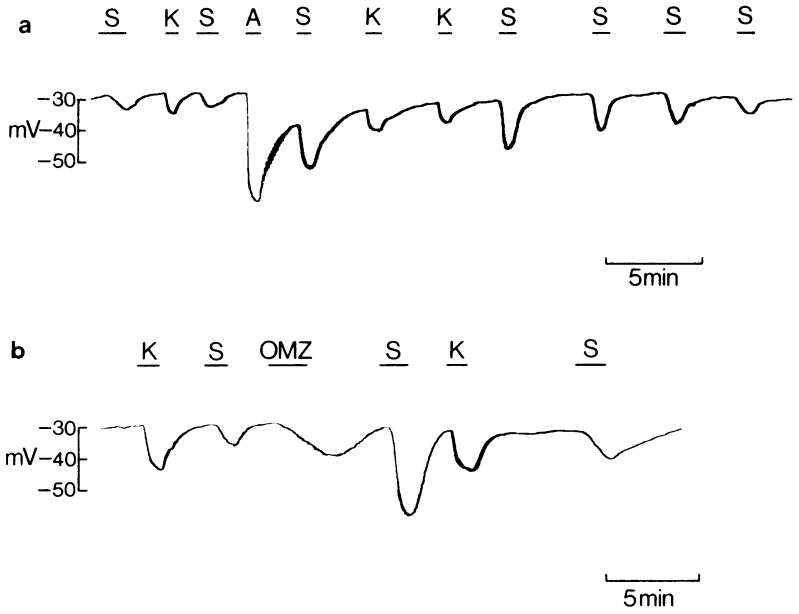

Figure 10 (a) Continuous record of the membrane potential of a cell in a liver slice bathed in a potassium and chloride-free solution (chloride replaced by isethionate, potassium by sodium). Brief re-introduction of potassium (6 mM, at K) caused hyperpolarization (see text). In contrast to the hyperpolarization caused by salbutamol (0.5 μM, at S), this response to potassium was no greater after (±)-amidephrine (20 μM, at A). (b) As in (a) but with oxymetazoline (1 μM, at OMZ) instead of (±)-amidephrine. From the same preparation, but 25 min later.

those α-agonists which cause prolonged potentiation act in a qualitatively different way from the other agents, and this requires further study.

It was also found that the adenine nucleotides ADP and ATP cause a transient dose-dependent increase in the membrane potential of guinea-pig liver cells. Although the mechanism of this hyperpolarization has yet to be examined, the fact that the response to these nucleotides was not increased by α-agonists suggests, albeit indirectly, that changes in cyclic AMP are not involved. Indeed, the additional finding that ADP and ATP can potentiate the response to a β-agonist suggests that the action of both nucleotides may have more in common with α- than with β-receptor activation. ATP-induced hyperpolarizations have also been described in smooth muscle by Tomita & Watanabe (1973), and these authors favour an explanation based on an increase in the potassium permeability of the membrane; the effects of ADP and ATP on membrane potential in the liver may possibly arise in the same way. Whatever the mechanism, the finding that compounds other than α-agonists are capable of potentiating the response to a β-agonist strongly suggests that the observed interactions involve not the α- and β-receptors *per se*, but one or more of the steps which follow their activation.

Since several of these steps are as yet only partly understood, further discussion is necessarily speculative. However, there are two pieces of evidence which suggest possible lines for such speculation to follow. Firstly, β-agonists increase the cyclic AMP content of guinea-pig liver slices (Osborn, 1975), and since the response to applied cyclic AMP itself is facilitated by α-agonists, it seems possible that potentiation reflects a change in the way in which the cell responds to an increase in the concentration of this nucleotide in the cytoplasm. Secondly, recent work by Haylett (1976) has shown that α-agonists (amidephrine and noradrenaline) cause a large though transient rise in the efflux of labelled calcium from guinea-pig and rabbit liver slices, isoprenaline being much less effective under normal conditions. Though the interpretation is still tentative, this α-mediated increase in calcium efflux may reflect a rise in the concentration of calcium ions in the cytoplasm. This increase in calcium ion concentration could in turn underlie the observed rise in potassium permeability, as has been described for other tissues, including erythrocytes, salivary glands, and several vertebrate and invertebrate neurones (see Berridge, 1975, for references).

Since the 'potentiated' membrane response to β-

activation is so similar to the α-action (in each instance, there is an increase of similar time course in membrane potential and potassium efflux—see Haylett & Jenkinson, 1973), it is tempting to suppose that when potentiation occurs, cyclic AMP is now able to increase cytoplasmic calcium, though by a different mechanism from that which operates during α-activation. In the absence of any direct evidence to indicate the source of this calcium it is clearly premature to advance detailed suggestions about the two postulated mechanisms for calcium release, or about their interactions. Nevertheless, it should be mentioned that in other tissues, conditions exist in which two 'cellular' calcium pools interact to give rise to an enhanced, even regenerative, calcium release (e.g. Fabiato & Fabiato, 1975). Also, both isoprenaline and externally applied cyclic AMP cause a much greater increase in ^{45}Ca efflux from guinea-pig liver slices when applied after an α-agonist (D.G. Haylett, D.H. Jenkinson & K. Koller, unpublished observations), which is in keeping with the above suggestions (see also Kroeger & Marshall, 1973).

Another possible explanation for potentiation could be envisaged if cyclic AMP were to exert a more direct effect on the ionic permeability of the liver cell, as has been suggested for other inexcitable tissues e.g. avian erythrocytes (see Gardner, Mensh, Kiino & Aurbach, 1975 for references). The relative inability of cyclic AMP (whether externally applied, or resulting from β-receptor activation) to affect membrane potential in untreated slices might then be explained if, as it seems reasonable to suppose, there is a basal level of phosphodiesterase activity which serves to keep down the concentration of cyclic AMP within the cells, and more particularly, at the inner surface of the membrane.

If activation of the α-receptors in some way depresses this basal phosphodiesterase activity, subsequent application of a β-agonist (or of cyclic AMP) could result in an increase in cyclic AMP at the membrane, and hence in a rise in potassium permeability, and membrane potential. Since potentiation of β-mediated responses can be seen with both α-agonists and the adenine nucleotides ADP and ATP it is difficult to imagine that all these agents could directly inhibit phosphodiesterase, but an indirect effect (e.g. through increased levels of intracellular calcium) might be envisaged. In principle, this should have been testable by the application of selective phosphodiesterase inhibitors which on such a scheme would be expected to increase greatly the responses to cyclic AMP and to β-agonists, and at the same time to reduce or abolish potentiation, as defined in the present work. Unfortunately, the results obtained with phosphodiesterase inhibitors were complex; though the predicted rise in the response to β-agonists was observed, it was quickly followed by a long-lasting inhibition, of unknown origin. The experiments did not therefore allow us to distinguish between these possible explanations for potentiation, which are indeed only two of several that can be proposed on the basis of the diverse interactions that occur between intracellular calcium and the enzymes concerned in the formation, action and destruction of cyclic nucleotides (for reviews, see Rasmussen & Goodman, 1975; Berridge, 1975). More will have to be known about these interactions, and about the direct and indirect effects of cyclic nucleotides on liver cell membranes, before the point can be settled.

Possible implications

Although we have not tested whether potentiation occurs in other *in vitro* situations or *in vivo*, there are interesting parallels between the present findings and some recent reports on the effects of sympathomimetic amines on plasma potassium in dogs. It has long been known that adrenaline causes a biphasic change in plasma potassium in several species, an early rise being followed by a prolonged fall. While it is clear that the initial rise is mainly due to a net loss of potassium from the liver (D'Silva, 1936; 1937; Todd & Vick, 1971; Vick, Todd & Luedke, 1972), there is conflicting evidence on the type of receptors concerned in this action. The finding (e.g. Todd & Vick, 1971) that the hyperkalaemia in the dog can be abolished by α-blocking agents suggest that it is α-mediated, as in the cat (Ellis & Beckett, 1963). However, this has not been easy to reconcile with the parallel observation, also in the dog, that the initial hyperkalaemia is either partially (Grassi, de Lew, Cingolani & Blesa, 1971) or almost completely (Todd & Vick, 1971) inhibited by propranolol (though see also Castro-Tavares, 1976). A further puzzle is that in this species combined administration of low doses of an α- and a β-agonist (phenylephrine and isoprenaline respectively) causes a 'super-additive hyperkalaemia' i.e. an increase in plasma potassium much greater than the sum of the separate responses to each drug (Todd & Vick, 1971; Castro-Tavares, 1975). These authors have favoured explanations based on β-mediated changes in blood flow; the increased loss of potassium is considered to result either from more effective 'washout' from the liver (Todd & Vick, 1971), or from increased hepatic hypoxaemia due to β-mediated opening of vascular shunts within the liver (Castro-Tavares, 1975). However, the present finding that simultaneous activation of α- and β-receptors caused a more than additive increase in membrane potential in an isolated preparation (e.g. Figures 2a,b and 8b) makes it tempting to seek at least a partial explanation of the *in vivo* findings in terms of an interaction at the level of the liver cells themselves. Should potentiation be found to occur in intact liver, or indeed in other situations, it is clear that receptor classification studies would require particular care. For

example, an α-adrenoceptor antagonist might be found to reduce the response to a β- as well as to an α-agonist (see Figure 3). The finding that α-agonists can sometimes potentiate a β-response also raises the more general point that the effects of adrenaline and noradrenaline (which activate both receptors) may not always be equivalent to the sum of the responses to selective activation of each receptor.

We are grateful to Professor J.W. Black, Dr D.G. Haylett and Dr R. Niedergerke for helpful discussions, and to Mrs Margaret Butler for skilful technical assistance. K.K. held an M.R.C. studentship during the course of the work. We would like to thank Mead Johnson, Ward & Blenkinsop, Allen & Hanbury, Burroughs Wellcome, Pfizer, Boehringer Ingelheim, William R. Warner and ICI for gifts of drugs mentioned in the Methods section.

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(Received June 23, 1976
Revised August 13, 1976.)