Br. J. Pharmac. (1983), **79**, 441–449

Cyclic adenosine 3′,5′-monophosphate and β-effects in rat isolated superior cervical ganglia

D.A. Brown & P.M. Dunn[1]

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX

1 Isoprenaline (0.01–1 μM) increased the amount of cyclic adrenosine 3′,5′-monophosphate (cyclic AMP) in rat isolated superior cervical ganglia by up to 10 times after 10 min application. Cyclic AMP levels returned to control values after 20 min washing.

2 Salbutamol, in concentrations (1–100 μM) that depolarized the ganglion and facilitated submaximal transmission, did not significantly raise ganglionic cyclic AMP levels.

3 The action of isoprenaline was antagonized by butoxamine (apparent $K_I \approx 0.14$ μM) and weakly by practolol (apparent $K_I \approx 9.1$ μM).

4 The effect of 0.1 μM isoprenaline was also inhibited 94% by 100 μM of the adenylate cyclase inhibitor, 9-(tetrahydro-2-furyl)adenine (SQ 22,536).

5 Exogenous dibutyryl cyclic AMP did not replicate the effects of isoprenaline on ganglionic d.c. potentials or submaximal transmission.

6 The phosphodiesterase inhibitors theophylline, isobutylmethylxanthine or 4-(3,4-dibutoxybenzyl)-2-imidazolidinone (Ro 20-1724) did not potentiate these electrical responses to isoprenaline.

7 The adenylate cyclase inhibitor, SQ 22,536, did not inhibit the electrical responses to isoprenaline.

8 It is concluded that available evidence does not support the view that the ganglion depolarization or facilitation of submaximal transmission in rat isolated ganglia produced by isoprenaline are likely to be mediated by cyclic AMP.

Introduction

β-Adrenoceptor agonists can produce a large (about 10 fold) increase in the amount of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in rat isolated superior cervical ganglia (Cramer, Johnson, Hanbauer, Silberstein & Kopin, 1973; Lindl & Cramer, 1975; Quenzer, Alkadhi & Volle, 1979; Brown, Caulfield & Kirby, 1979; Briggs, Whiting, Ariano & McAfee, 1982). The significance of this is unclear, since the principal effect of catecholamines previously detected in this tissue, postsynaptic hyperpolarization, depression of transmission and inhibition of Ca^{2+}-currents, were clearly mediated by α-receptors (Suzuki & Volle, 1978; Quenzer *et al.*, 1979; Brown & Caulfield, 1979; 1981; Horn & McAfee, 1979; McAfee, Heron, Whiting, Horn, Yarowsky & Turner, 1980; Adams & Galvan, 1981). However, we (Dunn, 1982; Brown & Dunn, 1983) have recently detected additional β-mediated 'excitatory' effects of catecholamines on rat superior cervical ganglion *in vitro*, comprising pre- and postsynaptic depolarization and, under conditions of reduced transmitter release, facilitation of submaximal transmission. The present experiments were undertaken to see if these effects could reasonably be attributed to the increased levels of cyclic AMP. Two forms of experiment have been tried. Firstly, the pharmacological characteristics of the β-receptor triggering the cyclic AMP elevation have been explored in more detail than hitherto, to see whether they match those responsible for the ganglion depolarization (which appear to conform to the β_2-subtype: see Brown & Dunn, 1983). Secondly, the effects of cyclic AMP and of other agents modifying cyclic AMP action or metabolism on the ganglion have been further compared with those of the catecholamines.

[1]Present address: Department of Physiology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, N.C. 27514, U.S.A.

Methods

Freshly-dissected superior cervical ganglia were obtained from rats anaesthetized with urethane and prepared for extracellular recording of ganglionic d.c. potentials and transmitted action potentials as described in the preceding paper (Brown & Dunn, 1983). For cyclic AMP measurements, which required larger numbers of ganglia, the ganglia were obtained rapidly from rats killed by decapitation, then cleaned of connective tissue and maintained in Krebs solution at 22°C under the same conditions as for electrical recording. Each ganglion was pre-incubated in 1.35 ml Krebs solution for 30 min. Drugs or Krebs solution (for controls) were then added in 0.15 ml Krebs solution, to give a total incubation volume of 1.5 ml per ganglion; antagonists were added to the pre-incubation mixture. After incubation the ganglia were transferred to 200 μl distilled water heated in a boiling water bath for 5 min and homogenized. The homogenate was then made up to 400 μl with distilled water and centrifuged at 400 *g* for 10 min at 4°C. Cyclic AMP in the supernatant was assayed by the protein binding method of Gilman (1970), using the following incubation mixture (total volume 100 μl): 40 μl sample, 10 μl phosphate/citrate buffer at pH 5.8; 10 μl [^{3}H]-cyclic AMP (0.5 pmol, 26 Ci mmol^{-1}) in distilled water; and 50 μl protein kinase (1 mg ml^{-1}) in 5 mM phosphate buffer at pH 7 containing 1 mg ml^{-1} bovine serum albumin. The assay mixture was incubated at 4°C for 2 h, then diluted with 1 ml ice-cold 20 mM phosphate buffer at pH 6 and vacuum filtered through pre-wetted millipore HAWP 0.45 μm filters. Incubation tubes were rinsed with a further 1 ml buffer, refiltered, and the filters washed twice with 4 ml cold buffer solution. Radioactivity on the filters was assayed by scintillation counting in 10 ml 'Aquasol' (N.E.N.) after dissolution in 2 ml 2-ethoxyethanol. For calibration, 1 to 20 pmol cyclic AMP was added instead of the ganglion extract; least-squares regression lines were fitted to the calibration curve and the cyclic AMP content of the samples found by substituting into the regression equation. Preliminary experiments showed that the amounts of cyclic AMP detected by this simplified assay procedure did not differ significantly from those obtained after separating cyclic AMP from cyclic guanosine monophosphate (cyclic GMP) using a Dowex AG1 column (cf. Brown *et al.*, 1979), presumably because cyclic GMP levels in ganglia are too low to interfere with the assay (see Quenzer, Patterson & Volle, 1980). Hence, column separation was not routinely used. No phosphodiesterase inhibitor was used in these experiments.

Cyclic AMP-dependent protein kinase, bovine serum albumin, theophylline hydrate, (−)-isoprenaline hydrochloride, isobutylmethylxanthine (IBMX), adenosine, adenosine mono-, di-, and triphosphates and adenosine 3′,5′-cyclic monophosphate (cyclic AMP) were obtained from Sigma; and [^{3}H]-cyclic AMP (26 Ci mmol^{-1}) was obtained from the Radiochemical Centre, Amersham. We are indebted to Roche Products Ltd for the gift of the phosphodiesterase inhibitor 4-(3,4-dibutoxybenzyl(-2-imidazolidinone (Ro 20-1724) and to Squibb for the gift of the adenylate cyclase inhibitors 9-(tetrahydro-2-furyl)adenine (SQ 22,536) and 9-benzyl-adenine (SQ 21,611).

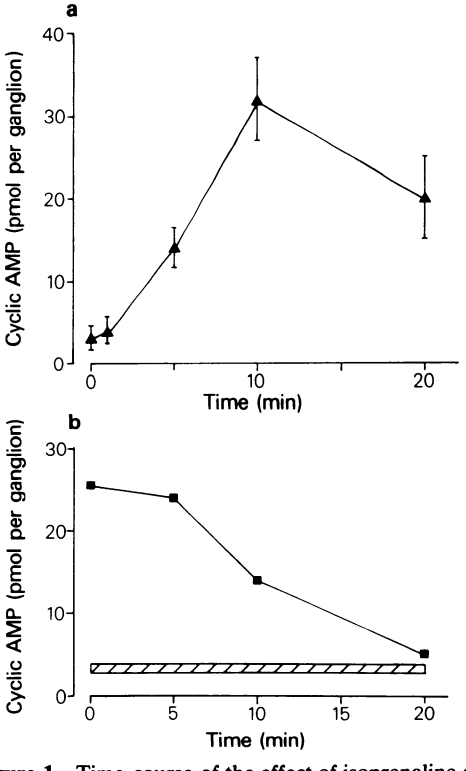

Figure 1 Time-course of the effect of isoprenaline on the amount of cyclic AMP in whole rat superior cervical ganglia *in vitro* at 22°C (a) Increase in cyclic AMP during exposure to 0.1 μM isoprenaline. Each point represents the mean level in 4 ganglia; bars show s.e.mean (b) Decline in cyclic AMP after washout of isoprenaline. Each point shows the mean of 2 ganglia removed from 0.1 μM isoprenaline after 10 min incubation and rinsed in normal Krebs solution for the time indicated. The hatched bar shows the range in 2 ganglia not exposed to isoprenaline solution and incubated for 30 min in normal Krebs solution. Ordinates: amount of cyclic AMP (pmol) per ganglion.

Results

Cyclic AMP measurements

Unstimulated ganglia contained an average of 3.0 ± 0.3 pmol cyclic AMP per ganglion (mean ± s.e.mean, $n = 32$). Addition of 0.1 μM isoprenaline increased the cyclic AMP content to 31.7 ± 4.9 pmol per ganglion (mean ± s.e.mean, $n = 4$) after 10 min (Figure 1a). This agrees with the 10 fold elevation after 15 min exposure to isoprenaline in the absence of a phosphodiesterase inhibitor previously found by Brown *et al.* (1979), although the absolute levels were some 3–4 times lower in the freshly-dissected ganglia used in the present study. After longer incubation the cyclic AMP level tended to fall. Control levels were regained some 20 min after removing isoprenaline.

The elevation of cyclic AMP after 10 min incubation increased with increasing concentrations of isoprenaline over the range 10 nM to 1 μM (Figure 2), again in agreement with Brown *et al.* (1979) and also with Quenzer *et al.* (1979). Thus about a 10 fold higher concentration was required to elevate cyclic AMP than that which depolarized the ganglion (interrupted line in Figure 2; cf. Figure 4 in Brown & Dunn, 1983).

Salbutamol, which is some 35 times less potent than isoprenaline in depolarizing the ganglion (Brown & Dunn, 1983) did not significantly elevate cyclic AMP levels at concentrations up to 100 μM (Figure 2). In a further test of salbutamol and isoprenaline, the effects of a single concentration of each agonist (1 μM and 30 nM respectively) which had previously been found to produce an equivalent submaximal depolarization of the ganglion were compared. After 10 min incubation, ganglionic cyclic AMP levels were (pmol per ganglion): controls 4.2 ± 0.7; 30 nM isoprenaline, 18.4 ± 2.9; 1 μM salbutamol, 7.9 ± 1.6 ($n = 4$ in each case). Thus, depolarizing concentrations of isoprenaline significantly elevated cyclic AMP levels whereas an equal depolarizing concentration of salbutamol did not.

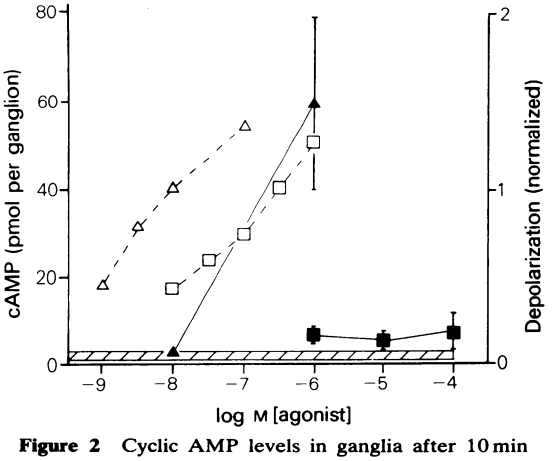

Figure 2 Cyclic AMP levels in ganglia after 10 min application of isoprenaline (▲) or salbutamol (■). Ordinates: amount of cyclic AMP in pmol per ganglion; abscissa scale: log molar concentration of agonist. Each filled point and bar shows the mean ± s.e.mean of 4 ganglia; the hatched bar shows the mean ± s.e.mean of 4 more ganglia incubated for an equivalent time in the absence of agonist. The open symbols (△, □) and interrupted lines show the mean depolarization produced by the two agonists expressed on a scale (right ordinates) relative to that produced by 10 nM isoprenaline (from Figure 4 of Brown & Dunn, 1983).

Brown *et al.* (1979) reported that the elevation of cyclic AMP produced by isoprenaline was antagonized by 1 μM propranolol. This effect was shared by the β_2-antagonist butoxamine but the β_1-antagonist practolol was much less active. Apparent K_I values from the dose-ratios were: butoxamine, 0.14 μM; practolol, 9.1 μM. The value for practolol agrees with that deduced from the pA_2 against isoprenaline-induced depolarization, whereas that for butoxamine is about 4 times greater (cf. Brown & Dunn, 1983).

A series of 9-substituted adenine derivatives can inhibit prostaglandin-activated adenylate cyclase activity in blood platelet lysate and intact platelets (Harris, Asaad, Phillips, Goldenberg & Antonaccio, 1979; Harris, Phillips, Goldenberg & Asaad, 1980). Two of these compounds 9-(tetrahydro-2-furyl)adenine (SQ 22,536) and 9-benzyl-adenine (SQ 21,611) were tested for inhibition of isoprenaline-induced cyclic AMP production. In these experiments 100 μM inhibitor was added 30 min before adding 0.1 μM isoprenaline, and cyclic AMP measured after a further 10 min incubation in isoprenaline plus inhibitor. SQ 22,536 reduced the isoprenaline-induced rise in cyclic AMP by 94% (Figure 4); SQ 21,611 was less effective and did not significantly reduce the effect of isoprenaline at 100 μM.

Cyclic nucleotides and ganglionic d.c. potentials

Dibutyryl cyclic AMP (db cyclic AMP 1 mM) produced variable and small changes in ganglionic d.c. potentials (Figure 5). In 3 (out of 8) ganglia db cyclic AMP produced a small (< 100 μV) slow hyperpolarization, like that previously described by Brown *et al.* (1979); in 3 more ganglia, db cyclic AMP produced a small depolarization; and in 2 ganglia no change in d.c. potential was recorded. All 8 ganglia were depolarized by isoprenaline, as previously reported (Brown & Dunn, 1983). Both hyperpolarizing and depolarizing responses to db cyclic AMP were blocked by theophylline (0.1 to 0.3 mM), suggesting that they were mediated by an effect on external

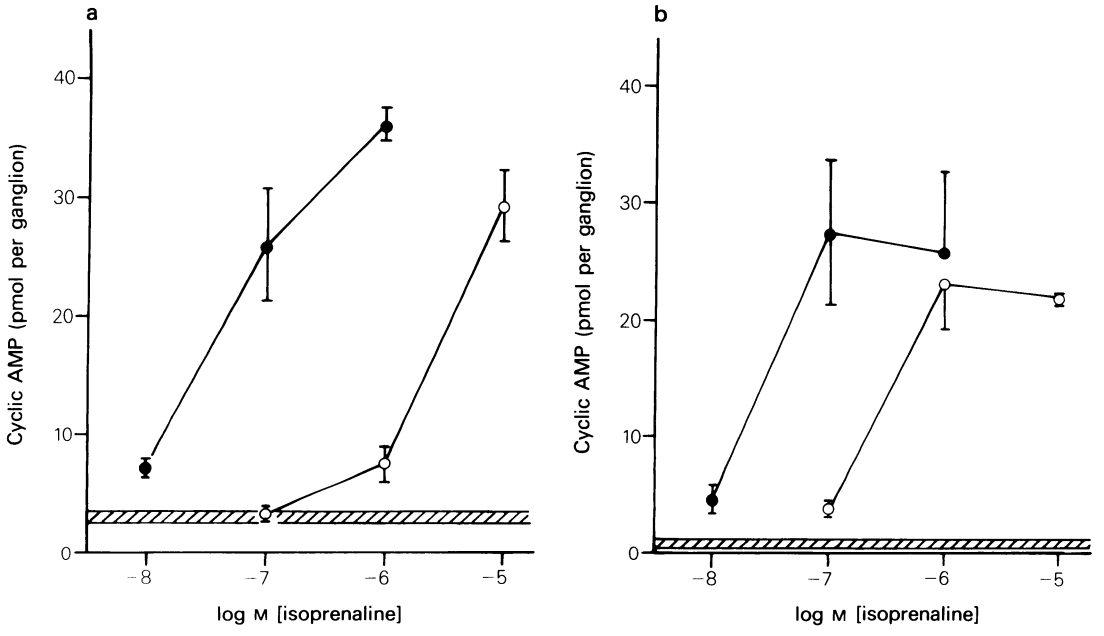

Figure 3 Antagonism of isoprenaline-induced elevation of cyclic AMP by (a) 100 μM butoxamine and (b) 10 μM oractolol. Each point, with bars, represents the mean ± s.e.mean of 4 ganglia incubated in the absence (●) or presence (○) of antagonist. Isoprenaline was added for 10 min; antagonist was added 30 min before isoprenaline. Hatched bars show mean ± s.e.mean of 4 ganglia not exposed to isoprenaline.

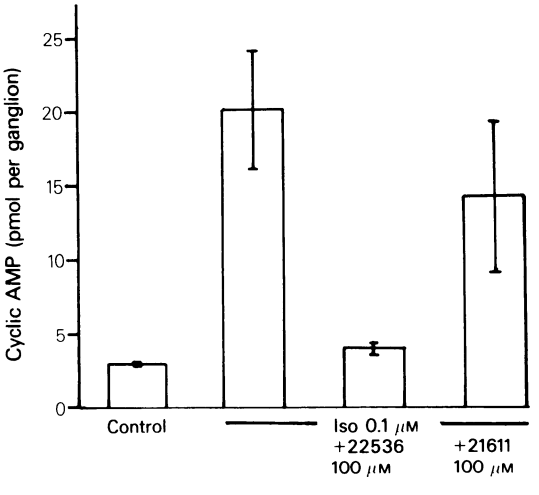

Figure 4 Effect of the adenylate cyclase inhibitors SQ 22,536 and SQ 21,611 on the elevation of cyclic AMP following 10 min application of 0.1 μM isoprenaline. The adenylate cyclase inhibitors were added 30 min before adding isoprenaline. Each column shows the mean ± s.e.mean of 4 ganglia; the control shows the cyclic AMP level in 4 ganglia not exposed to isoprenaline.

adenosine receptors as previously proposed (Brown *et al.*, 1979; see also McAfee *et al.*, 1980).

Theophylline had little effect on the depolarizing response to isoprenaline at concentrations < 0.3 mM; at 1 mM the response was reversibly and consistently reduced by about 40% (Figure 6a). This effect was duplicated by another xanthine derivative, isobutylmethylxanthine, at a 10 fold lower concentration (Figure 6b). In a single test, the structurally-unrelated phosphodiesterase inhibitor Ro 20-1724 (Sheppard, Wiggan & Tsien, 1972) had no clear effect at a concentration (100 μM) previously found to increase resting and stimulated cyclic AMP levels in ganglia (Kalix, McAfee, Schorderet & Greengard, 1974) (Figure 6c).

As described above, the isoprenaline-induced elevation of ganglion cyclic AMP can be inhibited by SQ 22,536 (cf. Figure 4). In contrast, no consistent or significant depression of the depolarizing response to isoprenaline could be detected after 20–30 min perfusion with 100 μM SQ 22,536 (Figure 7).

Faciliation of submaximal transmission

When transmitter release is reduced by altering the Ca/Mg ratio of the perfusion fluid, isoprenaline in-

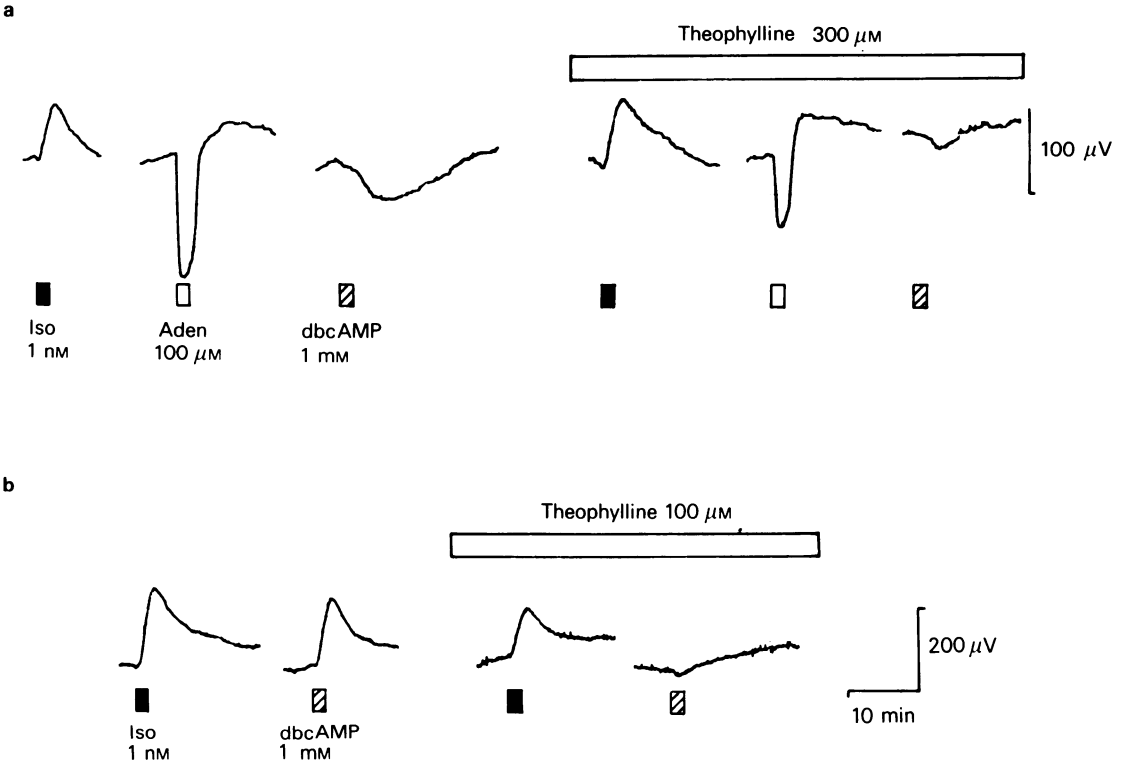

Figure 5 Ganglionic d.c.-potential changes produced by isoprenaline (Iso), dibutyrul cyclic AMP (dbcAMP) and adenosine (Aden), before and after adding theophylline to the perfusion fluid. An upward deflexion from baseline indicates ganglion depolarization. The two records (**a** and **b**) are from different ganglia. See Brown & Dunn (1983) for technical details.

creases the amplitude of the compound action potential evoked by single preganglionic nerve stimuli (Brown & Dunn, 1983). As shown in Figure 8, this effect was not replicated by db cyclic AMP: instead, 1 mM db cyclic AMP (or adenosine and its mono, di- and triphosphates, at 100 μM) further depressed transmission. This depression was inhibited by theophylline (100 μM), after which db cyclic AMP produced a long-lasting facilitation of transmission.

At concentrations $> 100\,\mu M$, theophylline or IBMX alone tended to facilitate transmission but did not augment the effect of isoprenaline. Ro 20-1724, at $< 10\,\mu M$, did not affect the action of isoprenaline; above 10 μM, it severely depressed transmission.

The adenylate cyclase inhibitor SQ 22,536 (100 μM) affected neither submaximal transmission nor the facilitation produced by isoprenaline (Figure 9).

Discussion

The hypothesis tested in these experiments is that the depolarization and transmission facilitation in rat isolated superior cervical ganglia produced by isoprenaline stems from the previously-reported elevation of ganglionic cyclic AMP. The results do not substantiate this hypothesis.

Firstly, the receptors responsible for the depolarization (and facilitation) appear to conform to the 'β_2'subclass of β-receptors, and hence are readily activated by salbutamol (Brown & Dunn, 1983). In contrast, salbutamol, unlike isoprenaline, did not significantly raise ganglionic cyclic AMP levels. Since the concentrations of isoprenaline required to elevate cyclic AMP were about ten times greater than those producing a depolarization, it might be argued that there is a considerable 'spare receptor' capacity

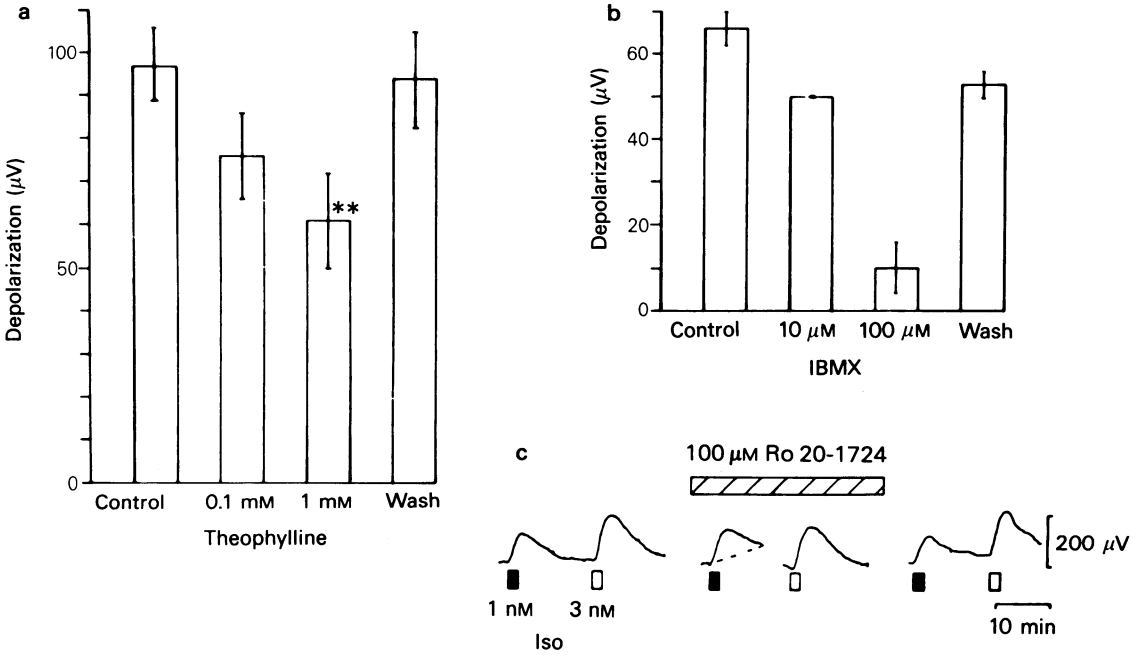

Figure 6 Effects of (a) theophylline, (b) isobutylmethylxanthine (IBMX) and (c) Ro 20-1724 on ganglion depolarizations produced by isoprenaline. The histograms in (a) and (b) show averaged responses to 1 nM isoprenaline in 2 separate ganglia before and during application of the phosphodiesterase inhibitor and after 30 min washing; each bar is the mean ± s.e.mean of at least 3 responses obtained at intervals > 15 min. (Responses were averaged because a near-threshold concentration of isoprenaline was used, to facilitate the detection of any potentiation, and hence the responses were very small.) The records in (c) show representative responses to 1 and 3 nM isoprenaline (Iso) before, during and after perfusion with 100 μM Ro 20-1724. Note the absence of any clear change in amplitude or time-course of the isoprenaline effect.

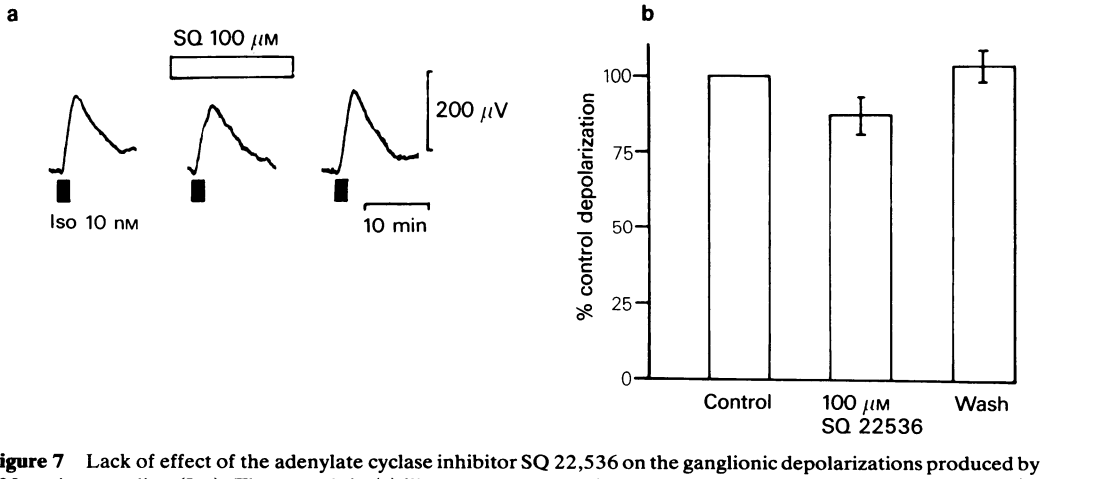

Figure 7 Lack of effect of the adenylate cyclase inhibitor SQ 22,536 on the ganglionic depolarizations produced by 100 nM isoprenaline (Iso). The records in (a) illustrate representative responses to isoprenaline in a single ganglion before, during and after adding 100 μM SQ 22,536 to the perfusion fluid; the histogram (b) shows mean responses in 3 experiments, ± s.e.mean, expressed as a percentage of the average response obtained before adding the cyclase inhibitor.

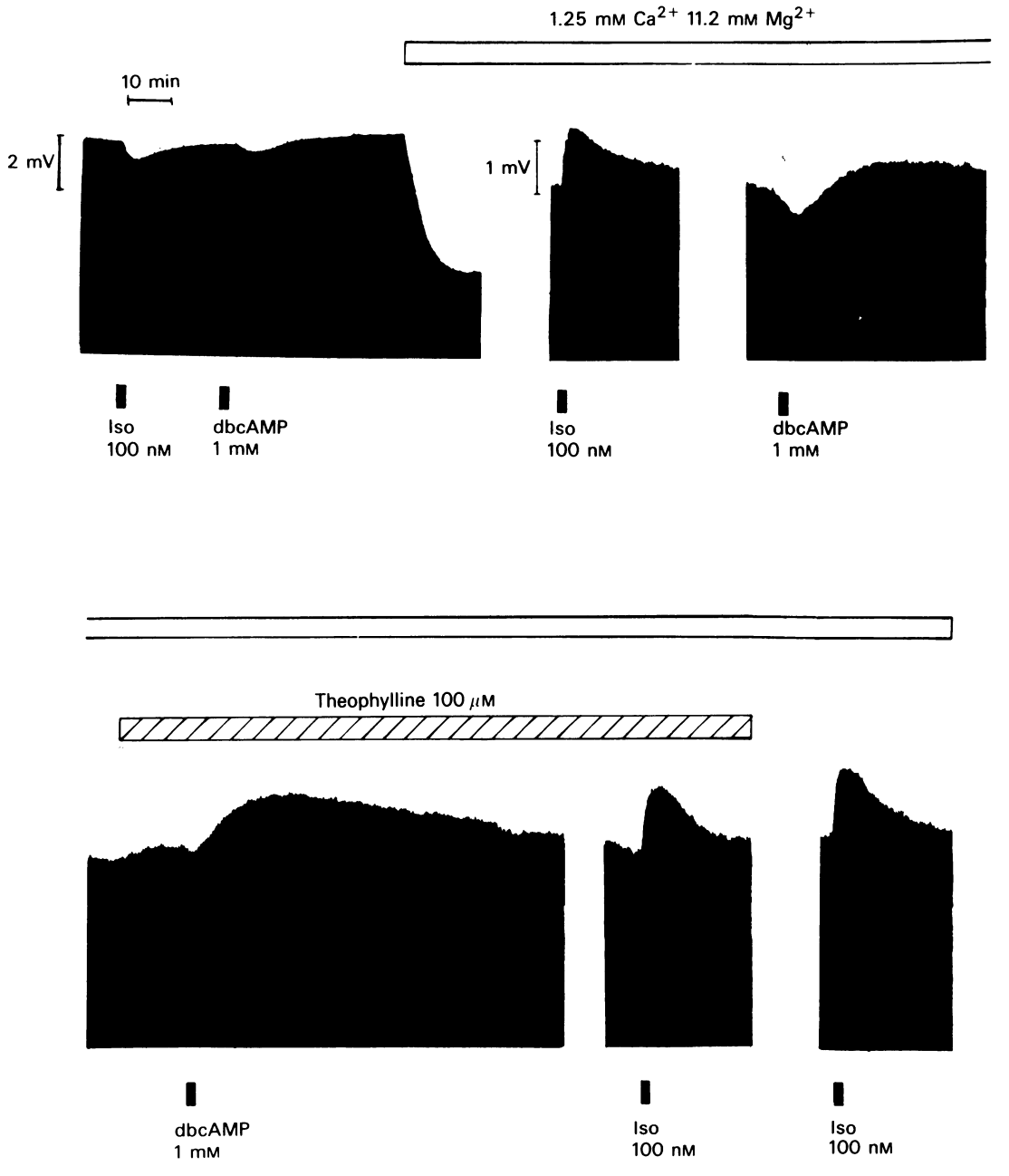

Figure 8 Effects of 100 nM isoprenaline (Iso), 1 mM dibutyryl cyclic AMP (dbcAMP) and 100 μM theophylline on the amplitude of the compound action potential recorded from an isolated ganglion following single supramaximal preganglionic stimuli at 0.2 Hz (see Brown & Dunn, 1983, for technical details). After an initial period of perfusion with normal Krebs solution (during which isoprenaline and db cyclic AMP produced a small reduction in evoked action potential amplitude), the perfusion fluid was changed to one containing 1.25 mM [Ca^{2+}] and 11.2 mM [Mg^{2+}] for the remainder of the experiment (indicated by the open bar). This depressed transmission by ~ 60%, so the recorder gain was doubled. Isoprenaline now facilitated transmission, whereas db cyclic AMP produced an initial depression followed by a slight, prolonged facilitation. Addition of theophylline (shaded bar, lower record) did not affect the response to isoprenaline but prevented the initial depressant action of db cyclic AMP.

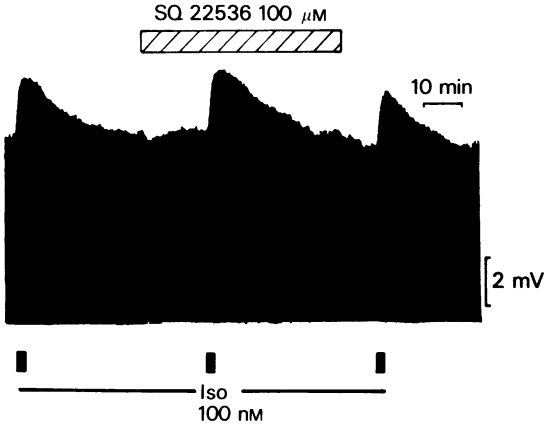

Figure 9 Lack of effect of the adenylate cyclase inhibitor SQ 22,536 (100 μM) on the potentiation of transmission by 100 nM isoprenaline (Iso). Transmission was recorded in a 1.25 mM $[Ca^{2+}]$ + 11.2 mM $[Mg^{2+}]$ solution, as in Figure 8.

in the system (as in some other adenylate cyclase-triggered responses to catecholamine: see Levitzki, 1976) and that consequently the amount of cyclic AMP needed to generate a depolarization following activation of adenylate cyclase by salbutamol was below the detectable threshold. This argument does not seem tenable because this difference between isoprenaline and salbutamol persisted when cyclic AMP levels were measured after application of concentrations which produced equal submaximal ganglion depolarizations. Since such depolarizations are antagonized with equal facility by β-receptor blocking agents and therefore can be attributed to an action on the same receptors (see Brown & Dunn, 1983), the most plausible conclusion is that the observed elevation of cyclic AMP is triggered via different receptors from those responsible for the ganglion depolarization. These 'cyclic AMP' receptors resemble β_2-receptors in terms of their relative susceptibility to the antagonists butoxamine and practolol, but not in their insensitivity to salbutamol.

Secondly, external application of dibutyrl cyclic AMP did not replicate the electrical effects of isoprenaline, nor did phosphodiesterase inhibitors potentiate the effects of isoprenaline. This is, of course, somewhat inconclusive because (a) there is no way of knowing whether appropriate intracellular concentrations were obtained and (b) these compounds can exert other effects, such as activation or inhibition of external adenosine receptors (see Brown *et al.*, 1979, and McAfee *et al.*, 1980, for further discussion of this point). External application of cyclic AMP or its derivatives has usually been observed to produce either a ganglion hyperpolarization (McAfee & Greengard, 1972; Machova & Kristofova, 1973; Brown *et al.*, 1979), or no clear effect (Dun, Kaibara & Karczmar, 1977; Dun & Karczmar, 1977; Gallagher & Shinnick-Gallagher, 1977; Busis, Weight & Smith, 1978), though a depolarization has been reported by Akasu & Koketsu (1977) in frog ganglia and by Hsu & McIsaac (1978) in rat ganglia. Such a depolarization was occasionally observed in the present experiments, but this was antagonized by theophylline suggesting that, like the previously-observed hyperpolarization (Brown *et al.*, 1979) it resulted from activation of external adenosine receptors. A depolarization following intracellular application of cyclic AMP has been reported by Gallagher & Shinnick-Gallagher (1977) but not by Kobayashi, Hashiguchi & Ushiyama (1978). The initial depression and subsequent prolonged facilitation of submaximal transmission produced by dibutyryl cyclic AMP is probably presynaptic in origin: Kuba, Kato, Kumamoto, Koketsu & Hirai (1981) have reported a corresponding initial reduction and later, sustained increase in the quantal content of the e.p.s.p. in frog ganglia. However, in rat ganglia this delayed facilitation was not replicated by isoprenaline.

Thirdly, external application of the adenylate cyclase inhibitor SQ 22,536 did not reduce either the depolarization or the facilitation of transmission produced by isoprenaline. Since the same concentration of inhibitor, applied in exactly the same manner, reduced the amount of cyclic AMP generated by the same concentration (0.1 μM) of isoprenaline by 94%, this seems to provide rather strong evidence that the generation of cyclic AMP is not a necessary intermediary for these β-mediated electrical events.

An alternative conclusion might be that the facilitatory effect of isoprenaline stems from a restricted pool of cyclic AMP which forms a very small proportion ($<5\%$) of the total measured during isoprenaline application, and which is insensitive to SQ 22,536. This is not totally implausible, because recent immunohistochemical experiments (Ariano, Briggs & McAfee, 1982) show that most of the resting cyclic AMP-immunofluorescence, and most of the increased immunofluorescence induced by isoprenaline, was located in satellite (neuroglial) cells, but that isoprenaline also caused the appearance of additional immunofluorescence in a small number of ganglionic neurones (see also Kebabian, Bloom, Steiner & Greengard, 1973; Otten, Mueller, Oesch & Thoenen, 1974). It would be useful to know whether the depolarization produced by isoprenaline were likewise restricted to a small proportion of the ganglion cells, though the next step, that of ascertaining whether cyclic AMP was generated only in electrically-responsive neurones, would be rather difficult in intact ganglia.

This work was aided, in part, by a grant from the Medical Research Council, P.M.D. was an S.E.R.C. CASE student and we thank Pfizer (U.K.) Ltd. for their support.

References

ADAMS, P.R. & GALVAN, M. (1981). Noradrenaline reduces calcium currents in rat sympathetic neurones. *J. Physiol.*, **318,** 41-42P.

AKASU, T. & KOKETSU, K. (1977). Effects of dibutyryl cyclic adenosine 3',5' monophosphate and theophylline on the bullfrog sympathetic ganglion cells. *Br. J. Pharmac.*, **60,** 331-336.

ARIANO, M.A., BRIGGS, C.A. & McAFEE, D.A. (1982). Cellular localization of cyclic nucleotide changes in rat superior cervical ganglion. *Cell. molec. Neurobiol.*, **2,** 143-156.

BRIGGS, C.A., WHITING, G.J., ARIANO, M.A. & McAFEE, D.A. (1982). Cyclic nucleotide metabolism in the sympathetic ganglion. *Cell. molec. Neurobiol.*, **2,** 129-141.

BROWN, D.A. & CAULFIELD, M.P. (1979). Hyperpolarizing 'α'$_2$-adrenoceptors in rat sympathetic ganglia. *Br. J. Pharmac.*, **65,** 435-445.

BROWN, D.A. & CAULFIELD, M.P. (1981). Adrenoceptors in ganglia. In *Adrenoceptors and Catecholamine Action*, ed. Kunos, G. pp. 99-115. New York: Wiley.

BROWN, D.A., CAULFIELD, M.P. & KIRBY, P.J. (1979). Relation between catecholamine-induced cyclic AMP changes and hyperpolarization in isolated rat sympathetic ganglia. *J. Physiol.*, **290,** 441-451.

BROWN, D.A. & DUNN, P.M. (1983). Depolarization of rat isolated superior cervical ganglia mediated by β_2-adrenoceptors. *Br. J. Pharmac.*, **79,** 429-439.

BUSIS, N.A., WEIGHT, F.F. & SMITH, P.A. (1978). Synaptic potentials in sympathetic ganglia: are they mediated by cyclic nucleotides? *Science (N.Y.)*, **200,** 1079-1081.

CRAMER, H., JOHNSON, D.G., HANBAUER, I., SILBERSTEIN, S.D. & KOPIN, I.J. (1973). Accumulation of cyclic AMP induced by catecholamines in rat superior cervical ganglia *in vitro*. *Brain Res.*, **53,** 97-105.

DUN, N.J., KAIBARA, K. & KARCZMAR, A.G. (1977). Dopamine and adenosine-3',5'-monophosphate responses of single mammalian neurons. *Science, N.Y.*, **197,** 778-780.

DUN, N.J. & KARCZMAR, A.G. (1977). A comparison of the effects of theophylline and cyclic adenosine 3',5'-monophosphate on the superior cervical ganglion of the rabbit by means of the sucrose-gap method. *J. Pharmac. exp. Ther.*, **202,** 89-96.

DUNN, P.M. (1982). β-Adrenoceptor-mediated responses of rat superior cervical ganglia *in vitro*. *Br. J. Pharmac.*, **75,** 17P.

GALLAGHER, J.P. & SHINNICK-GALLAGHER, P. (1977). Cyclic nucleotides injected intracellularly into rat superior cervical ganglion cells. *Science, (N.Y.)*, **189,** 851-853.

GILMAN, A.G. (1970). A protein binding assay for adenosine 3',5'-cyclic monophosphate. *Proc. natn. Acad. Sci., U.S.A.*, **67,** 305-312.

HARRIS, D.N., ASAAD, M.M., PHILLIPS, M.B., GOLDENBERG, H.J. & ANTONACCIO, M.J. (1979). Inhibition of adenylate cyclase in human platelets by 9-substituted adenine derivatives. *J. cycl. nucl. Res.*, **5,** 125-134.

HARRIS, D.N., PHILLIPS, M.B., GOLDENBERG, H.J. & ASAAD, M.M. (1980). Inhibition of adenylate cyclase and adenosine-3',5'-monophosphate phosphodiesterase. In *Enzyme Inhibitors as Drugs*, ed. Sandler, M. pp. 127-146. London: MacMillan.

HORN, J.P. & McAFEE, D.A. (1979). Norepinephrine inhibits calcium-dependent potentials in rat sympathetic neurones. *Science (N.Y.)*, **204,** 1233-1235.

HSU, S.Y. & McISAAC, R.J. (1978). Effects of theopylline and N^6-O^2-dibutyryl adenosine-3',5'-monophosphate on sympathetic ganglionic transmission in rats. *J. Pharmac. exp. Ther.*, **205,** 91-103.

KALIX, P., McAFEE, D.A., SCHORDERET, M. & GREENGARD, P. (1974). Pharmacological analysis of synaptically-mediated increase in cyclic AMP in rabbit superior cervical ganglion. *J. Pharmac. exp. Ther.*, **188,** 676-687.

KEBABIAN, J.W., BLOOM, F.E., STEINER, A.L. & GREENGARD, P. (1973). Neurotransmitters increase cyclic nucleotides in postganglionic neurons: immunocytochemical demonstration. *Science, N.Y.*, **190,** 157-159.

KOBAYASHI, H., HASHIGUCHI, T. & USHIYAMA, N.S. (1978). Post-synaptic modulation of excitatory process in sympathetic ganglia by cyclic AMP. *Nature.*, **271,** 268-270.

KUBA, K., KATO, E., KUMAMOTO, E., KOKETSU, K. & HIRAI, K. (1981). Sustained potentiation of transmitter release by adrenaline and dibutyryl cyclic AMP in sympathetic ganglia. *Nature*, **291,** 654-656.

LEVITZKI, A. (1976). Catecholamine receptors. In *Receptors and Recognition*, Series A, Volume 2, ed. Cuatrecasas, P. & Greaves, M.F. pp. 201-229. London: Chapman Hall.

LINDL, T. & CRAMER, H. (1975). Evidence against dopamine as the mediator of the rise in cyclic AMP in the superior cervical ganglion of the rat. *Biochem. biophys. Res. Comm.*, **65,** 731-739.

MACHOVA, J. & KRISTOFOVA, A. (1973). The effect of dibutyryl cyclic AMP, dopamine and aminophylline on ganglionic surface potential and transmission. *Life Sci.*, **13,** 525-535.

McAFEE, D.A. & GREENGARD, P. (1972). Adenosine 3',5'-monophosphate: electrophysiological evidence for a role in synaptic transmission. *Science (N.Y.)*, **178,** 310-312.

McAFEE, D.A., HENON, B.K., WHITING, G.J., HORN, J.P., YAROWSKY, P.J. & TURNER, D.K. (1980). The action of cyclic AMP and catecholamines in mammalian sympathetic ganglia. *Fedn Proc.*, **39,** 2997-3002.

OTTEN, U., MUELLER, R.A., OESCH, F. & THOENEN, H. (1974). Location of an isoprenaline-responsive cyclic AMP pool in adrenergic nerve cell bodies and its relationship to tyrosine 3-mono-oxygenase induction. *Proc. natn. Acad. Sci., U.S.A.*, **71,** 2217-2220.

QUENZER, L.F., PATTERSON, B.A. & VOLLE, R.L. (1980). The cyclic nucleotide content of the rat superior cervical ganglion. *J. Pharmac. exp. Ther.*, **215,** 297-303.

QUENZER, L.F., YAHN, D., ALKHADI, K. & VOLLE, R.L. (1979). Transmission blockade and stimulation of ganglionic adenylate cyclase by catecholamines. *J. Pharmac. exp. Ther.*, **208,** 31-36.

SHEPPARD, H., WIGGAN, G. & TSIEN, W.H. (1972). Structure-activity relationships for inhibitors of phosphodiesterase from erythrocytes and other tissues. *Adv. cyclic Nucleotide Res.*, **1,** 103-112.

SUZUKI, T. & VOLLE, R.L. (1978). Responses of the rat superior cervical ganglion *in vitro* to isoprenaline and bethanechol. *Naunyn-Schmiedebergs Arch. Pharmac.*, **304,** 15-20.

(Received October 18, 1983. Revised January 31, 1983.)