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EXPRESSION OF BETA$_2$-ADRENOCEPTORS MEDIATING CYCLIC AMP ACCUMULATION IN ASTROGLIAL AND NEURONAL CELL LINES DERIVED FROM THE RAT CNS

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Abstract—The effect of isoprenaline on cyclic AMP accumulation has been investigated in the rat neuronal cell line B50 and the rat astrocytoma cell line C6. Noradrenaline and isoprenaline stimulated cyclic AMP accumulation in both cell lines. Isoprenaline (0.5 μM; EC_{50} = 0.1 μM) produced a rapid ($T_{1/2}$ = 1.3 min) increase in [^{3}H]cyclic AMP accumulation in B50 cells while the response to isoprenaline (0.1 μM; EC_{50} = 0.01 μM) in C6 cells was somewhat slower ($T_{1/2}$ = 7.5 min). The response to 0.5 μM isoprenaline was antagonized by both propranolol (IC_{50} = 8.4 ± 1.6 nM; N = 3) and the β_2-selective antagonist ICI 118551 (IC_{50} = 2.1 ± 0.2 nM; N = 6). However, no attenuation of the response to isoprenaline (0.5 μM) was observed at concentrations of the β_1-adrenoceptor antagonist atenolol up to 10 μM (N = 3). In contrast, in C6 cells, which have previously been shown to possess β_1-adrenoceptors, atenolol inhibited isoprenaline-induced (0.1 μM) cyclic AMP accumulation (IC_{50} = 2.0 ± 0.5 μM; N = 6). Furthermore, the β_2-selective antagonist ICI 118551 was much less potent in the C6 cell line (IC_{50} = 0.2 ± 0.05 μM; N = 3) than in the B50 cells. In conclusion, the present data suggest that isoprenaline mediates cyclic AMP accumulation in the neuronal cell line via activation of β_2-adrenoceptors, while in the astrocytoma cell line the cyclic AMP response is mediated by β_1-adrenoceptors.

A common effect of chronic treatment of rats with antidepressant drugs is the desensitization and down-regulation of β-adrenoceptors linked to cyclic AMP formation in various brain regions [1–3]. The fact that these effects appear to develop over a similar time course to the therapeutic response to anti-depressant drugs in man has led to the modified biogenic amine theory of depression [1, 2]. At the present time, however, there is little information concerning the regulatory mechanism controlling the expression and desensitization of β-adrenoceptors in the central nervous system. This is mainly due to the heterogeneous cellular nature of brain preparations and the difficulty in determining the cellular location of the β-adrenoceptors under study.

Some success has been achieved with respect to cellular localization by analysing β-adrenoceptor function and number in CNS tissues in which specific cell types have been chemically removed, although the lesions induced rarely affect a single class of cells [4–6]. An alternative approach has been the isolation and cell culture (as primary cultures or cell lines) of specific populations of cells [7–10]. These latter studies have indicated that β_1-adrenocuptors are present on astrocytes and can markedly increase the formation of intracellular cyclic AMP [7, 8, 11–13]. Studies in rat cerebral cortical membranes, however, suggest that both β-adrenoceptor subtypes are present in the CNS in the ratio 65:35 (β_1:β_2) [14]. Thus, it is possible that these receptor subtypes are distributed differentially on different cell types. Autoradiographical studies, however, have failed to detect the presence of β-adrenoceptors on neurones in primary culture [10, 15].

In the present study we have investigated the effect of β-adrenoceptor stimulation on cyclic AMP formation in a neuronal cell line (B50) derived from the rat central nervous system [16]. These cells exhibit a number of properties characteristic of neurones. They possess an excitable membrane, extend processes in serum-free and dibutyryl cyclic AMP-containing media and express the neurotransmitter synthetic enzymes tyrosine hydroxylase and glutamic acid decarboxylase [16]. We now report the pharmacological characteristics of this β-adrenoceptor response in B50 cells and compare them with the properties of the β_1-adrenoceptor regulating cyclic AMP formation in an astrocytoma cell line (C6 cells) of rat CNS origin.

MATERIALS AND METHODS

Cell cultures. C6 and B50 cells obtained from the European Collection of Animal Cell Cultures (Porton Down, Salisbury, U.K.) were grown in Dulbecco's modified Eagles medium (DMEM) supplemented with 2 mM glutamine and 10% foetal calf serum at 37° under an atmosphere of 10% CO_2 in humidified air. Cells were grown in 75-cm^3 flasks (Costar) and passaged, without the use of proteolytic enzymes, every 3 days using a split ratio of 1:8. Experiments were performed in 24-well cluster dishes when cells had reached confluence (usually 3 days after seeding).

Accumulation of [^{3}H]*cyclic AMP.* Cyclic AMP accumulation was measured using a modification of the method described previously for brain slices [17]. Cells were incubated for 2 hr at 37° in 1 mL/well of DMEM medium containing foetal calf serum, glutamine and 0.08 μM (2 μCi/well) [^{3}H]adenine

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Table 1. Agonist EC_{50} values in B50 and C6 cells

Agonist	B50 cells		C6 cells	
	EC_{50} (μM)	(N)	EC_{50} (μM)	(N)
Isoprenaline	0.11 ± 0.02	(6)	0.013 ± 0.001	(3)
Noradrenaline	11 ± 3	(4)	0.19 ± 0.06	(3)

Values represent mean ± SE obtained in N separate experiments.

under an atmosphere of 10% CO_2 in humidified air. Prelabelled cell monolayers were then washed three times with 1 mL/well DMEM (minus serum) and incubated for 30 min in 1 mL/well of DMEM (minus serum) containing the phosphodiesterase inhibitor rolipram (0.1 mM) [17]. Agonists were then added in 10 μL of DMEM medium and the incubation continued for a further 10 min. Where appropriate, antagonist drugs were equilibrated with the cells for 30 min prior to agonist addition. Incubations were terminated by the addition of 50 μL of 10 M HCl. [^{3}H]Cyclic AMP in 0.95-mL aliquots of the supernatant layers was then isolated by sequential Dowex-alumina chromatography as described previously [17, 18].

Data analysis. Agonist concentration–response curves were fitted to a logistic equation using the non-linear regression program GraphPAD (ISI). The equation fitted was:

$$\text{Response} = \frac{E_{max} \times X^n}{(EC_{50})^n + X^n} + B,$$

where E_{max} is the maximal response, X is the concentration of agonist, EC_{50} is the concentration of agonist producing half maximal stimulation, B is the basal response and n is the Hill coefficient.

Antagonist inhibition curves were analysed using the same programme according to the expression:

$$\% \text{ of control response} = 100 - \frac{100 \times A^n}{(IC_{50})^n + A^n}$$

where IC_{50} is the concentration producing half maximal inhibition of the response to 0.1 μM (C6 cells) or 0.5 μM (B50 cells) isoprenaline and A is the antagonist concentration.

Apparent antagonist dissociation constants (K_D) were calculated using the expression:

$$X'/X^0 = 1 + IC_{50}/K_D,$$

where X' is the concentration of isoprenaline used in competition studies (0.1 μM for C6 cells or 0.5 μM for B50 cells) and X^0 is the concentration of isoprenaline calculated to yield the same response in the absence of antagonist as that produced at the IC_{50} concentration of antagonist using look-up tables (GraphPAD) based on the expression:

$$\text{Response} = \frac{100 \times X^n}{(EC_{50})^n + X^n}.$$

EC_{50} values were taken from Table 1 and the Hill coefficients were 1.4 and 1.05 for B50 and C6 cells, respectively. The calculated X'/X_0 ratios for the experiments in C6 and B50 cells were 9.1 and 5.4, respectively.

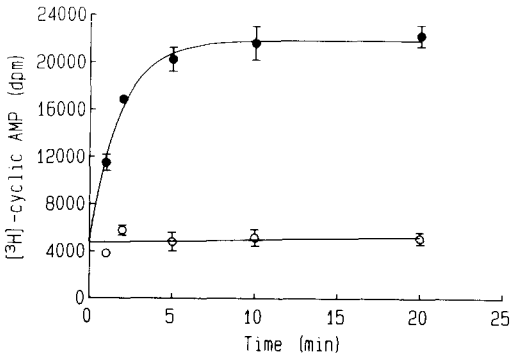

Fig. 1. Time course of the accumulation of [^{3}H]cyclic AMP induced by isoprenaline in B50 cells. Data were obtained in the presence (●) or absence (○) of isoprenaline (0.5 μM). Where appropriate isoprenaline or control medium was added at time zero. Values represent mean ± SE of quadruplicate determinations. Rolipram (0.1 mM) was present in all incubations. Similar data were obtained in three other experiments.

Unless otherwise stated, all values given in the text represent mean ± SE of N separate experiments.

Chemicals. Dowex 50W, H^+-form (200–400 mesh), neutral alumina (type WN-3), imidazole, (−)-noradrenaline bitartrate, (±)-isoprenaline hydrochloride, (±)-propranolol hydrochloride and atenolol were obtained from the Sigma Chemical Co. (Poole, U.K.). 8-[^{14}C]Cyclic AMP (sp. act. 42.4 mCi/mmol) was purchased from New England Nuclear (Stevenage, U.K.) and 8-[^{3}H]cyclic AMP from Amersham International (Bucks, U.K.). DMEM and foetal calf serum were obtained from Northumbria Biologicals (U.K.) and glutamine from Flow Laboratories (Herts, U.K.) Gifts of rolipram (Schering AG, Berlin, F.R.G.) and ICI 118551 (ICI Pharmaceuticals, Macclesfield, U.K.) are gratefully acknowledged.

RESULTS

Cyclic AMP formation in B50 cells

Isoprenaline (0.5 μM) produced a large and rapid increase in [^{3}H]cyclic AMP accumulation in B50 cells reaching a steady-state level within 5 min (Fig. 1). The mean $T_{1/2}$ obtained in four separate experiments was 1.3 ± 0.6 min. Concentration–response analysis of the the response to isoprenaline yielded an EC_{50} value of 0.1 μM (Table 1) and a slope parameter of

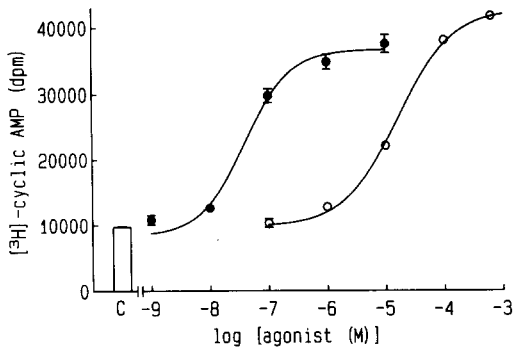

Fig. 2. Concentration–response curves obtained for isoprenaline (●) and noradrenaline (○) in B50 cells. The basal accumulation of [^{3}H]cyclic AMP is shown by the histogram marked C. Values represent mean ± SE of quadruplicate determinations in a single experiment. Similar data were obtained in three (noradrenaline) and five (isoprenaline) other experiments.

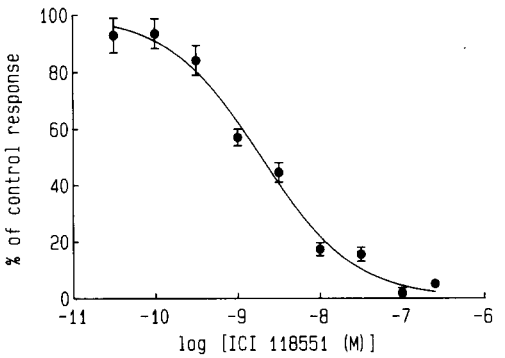

Fig. 3. Inhibition of isoprenaline (0.5 μM)-stimulated [^{3}H]cyclic AMP accumulation by the β_2-adrenoceptor antagonist ICI 118551 in B50 cells. Data are expressed as a percentage of the response to 0.5 μM isoprenaline, in the absence of ICI 118551, obtained in each experiment after subtraction of basal levels. Data represent the combined mean ± SE of quadruplicate determination obtained in each of six separate experiments.

Table 2. Effect of noradrenaline and isoprenaline combinations on cyclic AMP accumulation in B50 cells

Agonist	[^{3}H]Cyclic AMP (dpm)
−Rolipram	
Control	1776 ± 350
Isoprenaline (1 μM)	4785 ± 290
Noradrenaline (0.1 mM)	4813 ± 528
Isoprenaline (1 μM) + noradrenaline (0.1 mM)	4951 ± 185
+Rolipram (0.1 mM)	
Control	3191 ± 696
Isoprenaline (1 μM)	21,953 ± 752
Noradrenaline (0.1 mM)	21,927 ± 1060
Isoprenaline (1 μM) + noradrenaline (0.1 mM)	24,335 ± 3429

Values represent mean ± SE of six replicate determinations in a single experiment. Rolipram was added 30 min prior to agonist administration. Similar data were obtained in a second experiment.

1.4 ± 0.1 (N = 6). Noradrenaline produced a cyclic AMP response of similar size to that obtained with isoprenaline (Fig. 2; Table 2) but the EC_{50} was two orders of magnitude larger (Table 1). Combinations of maximally-effective concentrations of noradrenaline and isoprenaline produced no further increase in cyclic AMP levels, in the presence or absence of rolipram (Table 2), indicating the absence of any synergistic interactions such as those seen in intact rat brain slices [19, 20].

The response to isoprenaline (0.5 M) was potently antagonized by the β_2-selective antagonist ICI 118551 (Fig. 3; Ref. 21) and the non-selective antagonist propranolol (Table 3). However, the β_1-selective antagonist atenolol [22] was without significant effect at concentrations up to 10 μM (data not shown). In the presence of 10 μM atenolol, the response to 0.5 μM isoprenaline was 103.0 ± 7.8% (N = 4) of that obtained in the absence of the antagonist.

C6 astrocytoma cells

The cyclic AMP response to isoprenaline (0.1 μM) reached steady state levels over a much longer time course than that observed in B50 cells. The mean $T_{1/2}$ obtained in three experiments was 7.5 ± 0.4 min. The responses to both noradrenaline and isoprenaline were much larger in C6 cells with respect to basal levels (Fig. 4) than the corresponding responses in B50 cells (Fig. 2) and there were marked differences in the relative agonist potencies (Table 1). Thus, isoprenaline was one order of magnitude more potent than noradrenaline in C6 cells, but two orders of magnitude more potent in B50 cells. Combinations of maximally effective concentrations of noradrenaline (0.1 mM) and isoprenaline (0.1 μM) produced similar responses to those seen with isoprenaline and noradrenaline alone (data not shown; N = 4).

The response to 0.1 μM isoprenaline was antagonized by propranolol, ICI 118551 and atenolol

Table 3. Antagonist IC_{50} values and apparent dissociation constants

Antagonist	B50 cells		C6 cells	
	IC_{50} (nM)	K_D (nM)	IC_{50} (nM)	K_D (nM)
ICI 118551	2.1 ± 0.2	0.48 (6)	220 ± 50	27 (3)
Atenolol	>10,000	(3)	2000 ± 500	250 (6)
Propranolol	8.4 ± 1.6	1.9 (3)	78 ± 20	9.6 (3)

Values represent mean ± SE. The numbers of separate experiments conducted for each set of data are given in parenthesis. IC_{50} values were obtained from inhibition of the response to 0.1 μM (C6 cells) or 0.5 μM (B50 cells) isoprenaline. Rolipram (0.1 mM) was included in every incubation. Apparent K_D values were calculated from the IC_{50} values as described in Materials and Methods.

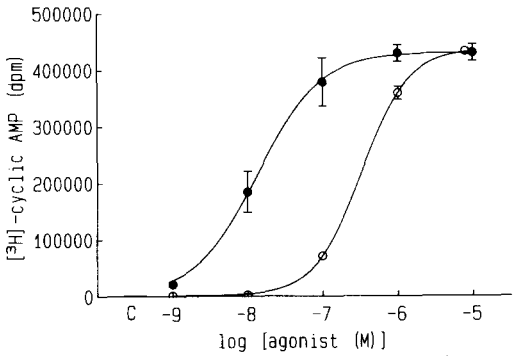

Fig. 4. Concentration–response curves for isoprenaline-(●) and noradrenaline- (○) stimulated cyclic AMP accumulation in C6 cells. The basal accumulation of [^{3}H]cyclic AMP is shown by the histogram marked C (1247 ± 70 dpm). Values represent mean ± SE of quadruplicate determinations in a single experiment. Similar data were obtained in two other experiments.

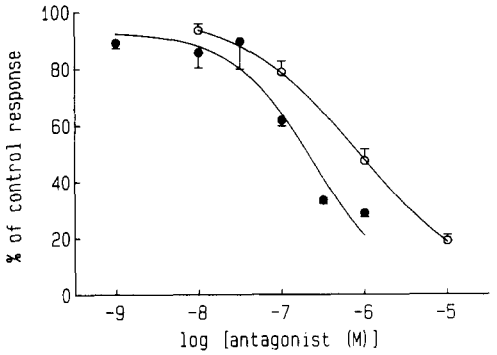

Fig. 5. Inhibition of isoprenaline (0.1 μM)-stimulated [^{3}H]cyclic AMP accumulation by ICI 118551 (●) and atenolol (○). Data are expressed as a percentage of the response to 0.1 μM isoprenaline, in the absence of antagonist, obtained in each experiment after subtraction of basal levels. Data represent the combined mean ± SE of quadruplicate determination obtained in each of three (ICI 118551) or six (atenolol) separate experiments.

(Table 3). Propranolol was the most potent antagonist, while the β_2-selective antagonist ICI 118551 was only one order of magnitude more potent than the β_1-selective antagonist atenolol (Fig. 5). These data contrast with the greater than 5000-fold difference in potencies of these two antagonists in B50 cells (Table 3).

DISCUSSION

The present results demonstrate that isoprenaline and noradrenaline can produce a marked stimulation of [^{3}H]cyclic AMP accumulation in a neuronal cell line derived from the rat CNS [16]. The response is rapid in onset ($T_{1/2}$ = 1.3 min) and has the pharmacological characteristics of a β_2-adrenoceptor-mediated response. Thus, the response to isoprenaline can be potently antagonized by the β_2-selective antagonist ICI 118551, while the β_1-selective adrenoceptor antagonist atenolol was without effect at concentrations up to 10 μM. The apparent K_D value (0.48 nM) for ICI 118551, calculated assuming competitive antagonism, agrees well with the values reported for this compound for other β_2-adrenoceptor-mediated responses (0.5 nM, guinea-pig uterus, [21]; 0.6 nM, bovine trachea, [23]).

The pharmacological characteristics of the cyclic AMP response to isoprenaline in B50 cells contrast markedly with those determined in C6 cells which, on the basis of binding studies, have been reported to contain predominantly the β_1-adrenoceptor subtype [12, 13]. Studies with ICI 118551 and atenolol on the cyclic AMP response to isoprenaline in the present study indicate that this functional response to β-agonists in this (C6) cell line is mediated via β_1-adrenoceptor activation. The estimated K_D values for these two compounds (ICI 118551, 27 nM; atenolol, 250 nM; Table 3) agree well with the values obtained for β_1-adrenoceptors (67 nM, [21]; 310 nM, [12] for ICI 118551 and atenolol, respectively).

A characteristic feature of the cyclic AMP response to noradrenaline in slices of rat cerebral cortex is the fact that both β- and α-adrenoceptors contribute to the final response [24, 25]. Thus, the direct effect of β-adrenoceptor stimulation on cyclic AMP formation is augmented by simultaneous activation of α-adrenoceptors [20, 25, 26]. However, unlike the situation in brain slices [20, 25], simultaneous addition of maximally-effective concentrations of isoprenaline and noradrenaline to B50 or C6 cells did not produce a greater elevation of cyclic AMP levels over that seen with either agonist alone. Furthermore, the equality of the maximal responses to isoprenaline and noradrenaline in each cell line

indicates that there is no α_2-adrenoceptor-mediated inhibition of adenylate cyclase activity present in the response to noradrenaline; unlike the situation in primary astrocytes in culture [7, 11, 27].

In summary, the present study has demonstrated that β-agonists can stimulate cyclic AMP accumulation in two cell lines of rat CNS origin via different β-adrenoceptor subtypes. The neuronal cell line (B50) possesses functional β_2-adrenoceptors while the astrocytoma cell line (C6) contains functional β_1-adrenoceptors. Preliminary studies indicate that the β-adrenoceptor-mediated cyclic AMP response to the neurotransmitter noradrenaline in both cell systems is not complicated by the presence of modulatory α-adrenoceptor responses (α_1- or α_2-). These two cell lines should therefore prove to be important model systems in which to study the processes involved in regulating the expression and desensitization of β_1- and β_2-adrenoceptors in cells derives from the central nervous system.

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