Biochemical Pharmacology, Vol. 29, pp. 3155–3161.

0006-2952/80/1201-3155 $02.00/0

PRESENCE OF HISTAMINE AND SEROTONIN RECEPTORS ASSOCIATED WITH ADENYLATE CYCLASE IN CULTURED CALF-AORTA SMOOTH MUSCLE CELLS

PHARMACOLOGICAL CHARACTERIZATION OF THE HISTAMINE RESPONSE

ANNE R. LUCHINS and MAYNARD H. MAKMAN*
Albert Einstein College of Medicine, Bronx, NY 10461, U.S.A.

(*Received* 14 *March* 1980; *accepted* 3 *June* 1980)

Abstract—Intact cells and homogenate preparations of several lines of cultured calf-aorta smooth muscle cells contained adenylate cyclase activity stimulated markedly (2- to 3-fold) by epinephrine, histamine and serotonin. Pharmacological studies indicated the separate and independent nature of these three receptor–adenylate cyclase systems. Only the epinephrine response could be blocked by propranolol or be desensitized by pretreating with epinephrine. The serotonin-stimulated adenylate cyclase appeared to differ from other serotonin receptor systems in that it was refractory to all of the classical serotonin antagonists tested, with the exception of methysergide. Characterization of the histamine receptor, with the use of various H_1 and H_2 histamine agonists and antagonists, demonstrated it to be exclusively of the H_2 type. In this system, impromidine, a reported potent H_2 agonist, behaved as a mixed agonist–antagonist with a strong inhibitory effect on histamine stimulation. The high sensitivity of the histamine H_2 stimulated adenylate cyclase in the cultured smooth muscle cells makes this a useful system for studies of regulation of the histamine receptor *in vitro* as well as for screening putative H_2 antagonists and agonists.

In most past attempts to characterize peripheral histamine and serotonin receptors, isolated organ systems were used and electrophysiological variables were examined. Using these methods, the effects of histamine and serotonin on muscle contraction have been investigated in a variety of species and organs, including the rat stomach fundus [1], rabbit aortic strips [1], guinea pig cardiac muscle [2], dog hepatic vascular beds [3], and dog saphenous vein strips [4]. The heterogeneous cellular composition of these systems makes the assignment of a receptor to a particular cell type and the definition of molecular events triggered by hormone or neurotransmitter stimulation much more difficult. A 'second messenger' role of cyclic nucleotides in mediating peripheral hormones and central neurotransmission has been proposed for a wide variety of systems. In this regard, it has been reported that histamine stimulates gastric secretion by increasing adenosine 3′,5′-cyclic monophosphate (cAMP) levels in the gastric mucosa via its interaction with H_2-type histamine receptors [5]. Similarly, histamine-induced alterations in cyclic nucleotide formation or levels appear to regulate cardiac [6] and tracheal [7] contractions.

Although cultures of cells derived from the central nervous system have been examined extensively for various neurotransmitter receptors associated with adenylate or guanylate cyclases, only a few of these studies have concerned histamine and serotonin receptors [8–10]. Very little work concerning these receptors has been carried out with cultures of cells other than those derived from the CNS [11]. The use of homogeneous cultured cell types with distinct numbers of specific receptors should simplify the study of receptors and their associated adenylate cyclase. Cultured calf-aorta smooth muscle cells have been compared to smooth muscle cells found *in situ* with respect to biochemical and morphological characteristics [12]. Although levels of certain enzymes as well as some morphological and physical characteristics are altered, these cultured cells nevertheless retain some degree of differentiation and, thereby, provide a useful system for studies of arterial pathophysiology [13], of glycoprotein biosynthesis [14], and of receptors for, and action of, epidermal growth factor (EGF) [15, 16]. In the present investigation, in a number of these cultured smooth muscle cell lines, we demonstrate the presence of three independent hormone receptors for histamine, serotonin, and epinephrine, each associated with an adenylate cyclase; we further report the partial characterization of the histamine and serotonin receptor systems.

MATERIALS AND METHODS

5-Hydroxytryptamine hydrochloride (serotonin), 6-hydroxytryptamine, *N*-acetyl-5 hydroxytryptamine, 5-methoxytryptamine hydrochloride, *N*-methyl tryptamine, tryptamine hydrochloride, histamine dihydrochloride, pyrilamine maleate, L-epinephrine bitartrate, DL-propranolol hydrochloride and 2-chloro-adenosine were purchased from the

* Author to whom correspondence should be addressed: Maynard H. Makman, Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, U.S.A.

Sigma Chemical Co. St. Louis, MO. 5-Hydroxy-*N*-methyl tryptamine oxalate and IBMX were purchased from the Aldrich Chemical Co. Inc., Milwaukee, WI. Methysergide maleate was obtained from Sandoz Pharmaceuticals, East Hanover, NJ. Cinanserin hydrochloride was obtained from E. R. Squibb & Sons, Inc., Princeton, N.J. Cyproheptadine hydrochloride was obtained from Merck, Sharp & Dohme, Rahway, N.J. Metiamide, dimaprit dihydrochloride, impromidone trihydrochloride solution, 2- and 4-methyl histamine diydrochloride, and cimetidine were provided by Smith, Kline & French Laboratories, Philadelphia, PA.

The various lines of calf-aorta smooth muscle cells (XI, XII, XV, XVI and 41b) were originally derived from the medial layer of the thoracic aorta of young male calves. Explants from these medial layers were grown in BME (Basal Modified Eagle Medium, Gibco, Grand Island, NY) supplemented with 10 per cent fetal calf serum (Flow Laboratories, Rockville, MD) and incubated at 37 ° in 95 per cent air–5 per cent CO_2. Cells were serially propagated in stationary culture in this medium and were subcultured by trypsinization in Puck's saline containing 0.5 per cent trypsin–0.5 mM EDTA, as described previously [13]. Cells could be subcultured continuously for sixty generations before ceasing to divide. To ensure an adequate source of a particular cell line, aliquots of cells of early generation (approximately 2×10^6 cells per ml of BME with 10 per cent fetal calf serum and 7.5 per cent dimethylsulfoxide) were frozen in liquid nitrogen. Studies were carried out with 4lb and XV cell lines, and data were pooled, unless otherwise indicated.

Studies of cAMP formation were done with intact cells as follows. Falcon plastic Petri dishes (60 mm diameter) were inoculated with 2.68×10^5 cells. The culture was grown to confluency (about 6 days) with or without the addition of new medium on day 4 (no difference in results). Experiments were performed on day 6 or 7 when confluent cultures contained approximately 2×10^6 cells and 0.2–0.5 mg protein. Before an experiment, the growth medium was removed by aspiration. The attached cells were gently washed two times with BME without serum and then incubated at 37° in 1.0 ml BME without serum, containing 0.25 mM 3-isobutyl-l-methylxanthine (IBMX), for 20 min. This was followed by incubation with drug and/or hormone. The term hormone is used loosely to describe all three biogenic amines, even though it is recognized that these amine effectors may not necessarily be hormones in the conventional sense. *In situ,* the thoracic-aorta smooth muscle may be affected by circulating amines as well as by amines released locally from nerve endings or mast cells. After the appropriate incubation period (5 min unless indicated), the medium was removed and 0.5 ml of 50 per cent acetic acid was added. The plates were cooled on ice and then scraped so that the suspension could be transferred to glass tubes (10 × 75 mm). An aliquot was removed for protein determination by the method of Lowry *et al.* [17]. The suspension was then centrifuged at 3000 *g* for 10 min to sediment cellular debris. Aliquots were removed, dried, and then assessed for cAMP content by a competitive binding assay in which ^{3}H-labeled cAMP (New England Nuclear Corp., Boston, MA) and a rat brain fraction containing protein kinase were used. This assay is a modification of the procedure of Gilman [18].

With homogenates, assays of adenylate cyclase activity were carried out as follows. Falcon T75 flasks were inoculated with 10^6 cells and grown to confluency in 6–7 days with replacement of medium every third day. Experiments were performed on confluent cultures containing approximately 8×10^6 cells and 2.4 mg protein. Before an experiment the growth medium was removed, and the attached cells were washed and then preincubated for 30 min at 37° in BME without serum. After preincubation, medium was aspirated and cells were lysed in 2 mM Tris–maleate buffer (pH 7.4) containing 0.8 mM EGTA.* Cells were scraped and transferred to a glass homogenizer in which they were processed by three or four strokes. Aliquots (25 μl) of the homogenate were then added to tubes containing other assay ingredients in 75 μl of 100 mM Tris–maleate buffer (pH 7.8). The total volume per tube, including testing drug, was 110 μl with (final concentration) 0.5 mM ATP, 0.05 mM GTP, 1.0 mM $MgCl_2$, 0.5 mM IBMX, and 0.3 mM EGTA. Basal cAMP levels were determined by subtracting cAMP values obtained in tubes containing no ATP from tubes containing ATP and no drug. Tubes were incubated for 5 min at 32° in a shaking water bath; the reaction was ended by placing the tubes in a boiling water bath for 3.5 min. The tubes were then centrifuged at 3000 *g* and aliquots were assessed for cAMP content in the same way as in the intact cell assay.

Inhibition constants (K_i) for compounds that antagonized the histamine- or serotonin-stimulated increase in cAMP formation were determined in a set of experiments where the concentration of the hormone was held constant and the concentration of the antagonist was varied. The K_i value was calculated from the relationship of Cheng and Prusoff [19]: $K_i = IC_{50}/[1 + S/K_a]$ where IC_{50} = concentration of antagonist required for 50 per cent inhibition of the agonist-stimulated increase in cAMP formation, S = concentration of agonist, and K_a = the equilibrium dissociation constant for agonist. As an approximation of K_a, the EC_{50}, (the concentration for half-maximal stimulation of cAMP formation by the agonist) has been substituted; this substitution was made by others as well as by us in previous studies [20–22].

RESULTS

Sensitivity of intact cells and homogenates to hormones

Five different calf-aorta smooth muscle cell lines were assessed for stimulation of adenylate cyclase activity by histamine, serotonin and epinephrine. In every cell line tested, adenylate cyclase was stimulated to an appreciable extent by each of these hormones. In the three cell lines studied repeatedly (both with continuous subculturing and with reassay

* EGTA = ethyleneglycol-bis-(β-aminoethylether) *N*, *N*',-tetra-acetate.

Table 1. Hormone-stimulated adenylate cyclase activity of various calf-aorta cell lines*

(A) Cell line	N	Basal level (pmoles cAMP/mg protein)	Increment above basal level (pmoles cAMP/mg protein) Serotonin (10 μM)	Histamine (10 μM)
4lb	3	29 ± 3	71 ± 13 (245%)	194 ± 49 (669%)
XV	4	25 ± 3	75 ± 29 (300%)	219 ± 36 (876%)
XVI	3	28 ± 8	332 ± 27 (1185%)	214 ± 56 (764%)
(B) Cell line	N	Basal activity [pmoles cAMP·(mg protein)$^{-1}$·5 min]	Increment above basal activity [pmoles cAMP·(mg protein)$^{-1}$·5 min] Serotonin (10 μM)	Histamine (10 μM)
4lb	4	36 ± 10	80 ± 37 (222%)	92 ± 34 (255%)
XV	10	48 ± 9	48 ± 12 (100%)	128 ± 27 (267%)
XVI	4	40 ± 8	75 ± 18 (188%)	82 ± 13 (205%)

* Cyclic AMP levels of intact cells (A) and adenylate cyclase activity in cell homogenates (B) were determined as described in the text. N = number of separate experiments, each performed with triplicate incubations for each condition studied. The percent stimulation above basal activity is shown in parentheses.

of cells stored for various times in liquid nitrogen), the pattern of hormonal responsiveness was maintained. These patterns occurred at various cell densities and throughout the life span of the culture (Table 1).

The changes in levels of cAMP following additions of various concentrations of histamine, serotonin and epinephrine to intact cells and homogenates are illustrated in Fig. 1. In both homogenates and intact cell preparations, significant stimulation of cAMP levels could be induced with hormone concentrations above 0.1 μM; at 1.0 μM each of the three hormones stimulated cAMP production 100–300 per cent over basal levels. In contrast, no stimulation of the adenylate cyclase occurred with dopamine, amphetamine,

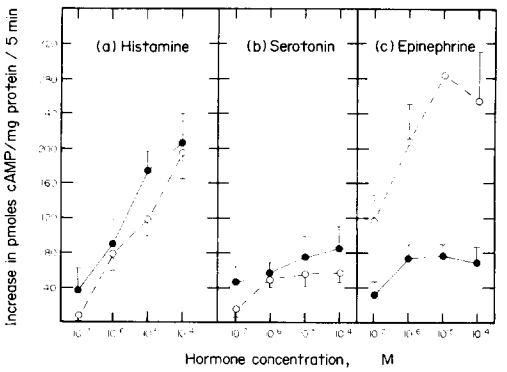

Fig. 1. Effects of various concentrations of histamine (A), serotonin (B), and epinephrine (C) on the level of cAMP in intact cells (●——●) and homogenates (○- - - -○). Values represent increments in cAMP above the basal levels that were 20 ± 3 pmoles/mg protein for intact cells and 44 ± 6 pmoles/mg protein for homogenates. Incubations with hormone were for 5 min. Assays were carried out as described in the text. For intact cell studies, the values are the means of fourteen, twelve, and three separate experiments for histamine, serotonin, and epinephrine respectively. For homogenate studies the values represent means of eleven, nine, and four separate experiments. Bar lines represent S.E.M.

metamphetamine, or octopamine at 10 or 100 μM concentrations. Ahn and Makman [23] have previously shown that metamphetamine and amphetmine, as well as dopamine and serotonin, can stimulate adenylate cyclase activity in homogenates of rhesus monkey anterior limbic cortex.

Stimulation by histamine was maximal at 100μM (data not shown). Maximum stimulation by serotonin and epinephrine was observed at approximately 10 μM. With intact cells, the concentration for half-maximal stimulation (EC_{50}) by histamine was 1.0 μM; the corresponding values for serotonin and epinephrine were, respectively, about 0.04 and 0.2 μM. Only with epinephrine was there a significantly larger stimulatory effect in homogenates than in intact cells. The reason for this phenomenon is unknown.

Time course of cAMP accumulation

The change with time of the cellular concentrations of cAMP in response to the addition of 10 μM histamine or serotonin is shown in Fig. 2. In each instance, the onset of increased cAMP generation occurred within 30 sec; the maximum generation occurred at 3 min with serotonin and at 20 min with histamine. The concentration of cAMP in control plates (no hormones added) did not change significantly during the 20-min incubation.

Response of receptor-mediated cAMP formation after prolonged exposure to hormone

Evidence that histamine and epinephrine receptors differ in their capacity to become desensitized is derived from hormone preincubation studies. After a 2-h preincubation with 0.1 mM epinephrine, intracellular cAMP levels returned to approximately the basal (control) level. However, when the cells were washed and treated once more with 0.1 mM epinephrine, the increment in cAMP level, measured 5 min later, was, on the average, 83 per cent less than the increment observed with 0.1 mM epinephrine in cells not pretreated. In contrast, preincubation with epinephrine caused a subsequent response

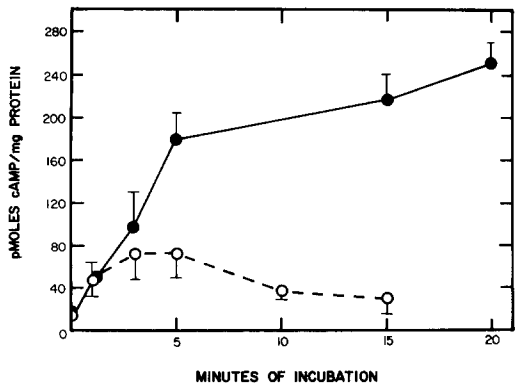

Fig. 2. Time course of stimulation of cyclic AMP formation by 10 μM histamine (●——●) and 10 μM serotonin (○- - - -○) in intact cells. Each value is the mean of three separate experiments, each carried out in triplicate as described in the text. The basal level of cAMP (13 ± 3 pmoles /mg protein) did not vary during the 20-minute incubation period. Bar lines represent S.E.M.

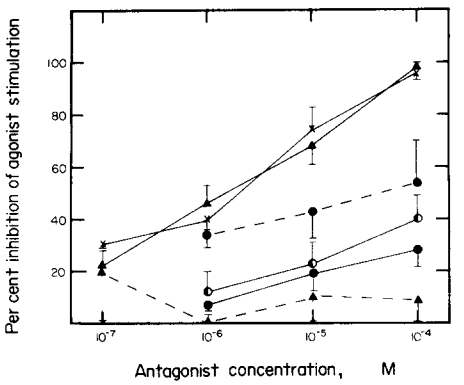

Fig. 3. Inhibition, by various concentrations of antagonists, of cAMP formation due to 10 μM histamine (——) or 10 μM serotonin (- - - -) in intact cells. Each point is the average of at least five incubations ± S.E.M. Key: metiamide (▲), methysergide (●), pyrilamine (◐), and cimetidine (X). Per cent inhibitions due to antagonist were calculated from the following equation: per cent inhibition =

$$\frac{[(\text{cAMP above basal with agonist}) - (\text{cAMP above basal with agonist and antagonist})]}{(\text{cAMP above basal with agonist})} \times 100.$$

K_i was calculated from the relationship $K_i = IC_{50}/[1 + S/EC_{50}]$ where IC_{50} = concentration of antagonist required for 50 per cent inhibition of the agonist-stimulated increase in cAMP formation, and S = concentration of agonist, EC_{50} (concentration of agonist to give half-maximal stimulation in the absence of antagonist) has been substituted for K_a (equilibrium dissociation constant) as described in Materials and Methods.

to histamine to be reduced by only 26 per cent. Similar studies involving preincubation with histamine indicated that the histamine receptor was resistant to desensitization. Thus, a 2-hr preincubation with 0.1 mM histamine led to only a 30 per cent reduction in a subsequent response to histamine, and to a negligible reduction in a subsequent response to 0.1 mM epinephrine.

Characterization of receptors with selective agonists and antagonists

Epinephrine receptor. Propranolol at 10 μM inhibited the response to 10 μM epinephrine by 95 ± 5 per cent without any inhibition of the response to 10 μM histamine or serotonin. The selective antagonism of the epinephrine stimulation of the adenylate cylcase by this β-adrenergic antagonist is supporting evidence for the presence of separate epinephrine receptor sites.

Serotonin receptor. In characterization studies of the serotonin receptor present on cultured smooth muscle cells, it was found initially that most classical peripheral serotonin antagonists were not able to block the stimulation of adenylate cyclase induced by serotonin. In contrast to the serotonin receptors that mediate contraction of rabbit aorta strips [24], the receptors of the cultured calf aorta were not antagonized significantly by pizotifen or cyproheptadine (tested at 10–100 μM in homogenates with 10 μM serotonin). Furthermore, the response to serotonin of intact-aorta cell cultures was not inhibited by cinanserin, molindone or lysergic acid diethylamide (LSD) (tested at 10–100 μM in the presence of 10 μM serotonin). Methysergide proved to be an exception since this classical serotonin antagonist at 10 μM produced a 40 per cent blockade of the serotonin response. Moreover, methysergide caused no inhibition of the epinephrine response (not shown) and appeared to be about 100-fold less potent as an inhibitor of histamine than of serotonin (Fig. 3).

To understand better the properties of this smooth muscle cell serotonin receptor, the stimulatory effects of various serotonin analogues were compared in both intact cells and homogenates. In both preparations, 5-hydroxy-*N*-methyl tryptamine and 5-methoxytryptamine were stimulatory (with one to two times the potency of serotonin at 10 and 100 μM), whereas tryptamine, *N*-methyl tryptamine, *N*-acetyl serotonin and 6 hydroxytryptamine gave no stimulation at 10 and 100 μM. These data demonstrate the relatively strict structural requirements for agonist activity. Thus, a hydroxyl or methoxy group on the 5-position of the indole ring system cannot be substituted, by such groups on the 6-position, and modification of the amine nitrogen by acetylation is also incompatible with activity.

The synergistic effects of adenosine and adenosine analogues such as 2-chloro-adenosine on some central monoamine response [20, 25] led us to assess whether stimulation by serotonin of cAMP formation in the calf-aorta cells could be potentiated by 2-chloro-adenosine. We found no enhancement of the response to 1 or 10 μM serotonin in the presence of 1 mM 2-chloro-adenosine. To insure that IBMX, which is routinely added to the intact cell assay, was not blocking this possible synergism [26], we tested the same concentrations of compounds in the absence of IBMX but found no potentiation.

Histamine receptor. The inhibition of the histamine sensitive adenylate cyclase was appraised using various receptor specific antagonists. Figure 3 illustrates the antagonism observed under experimental conditions where the concentration of histamine

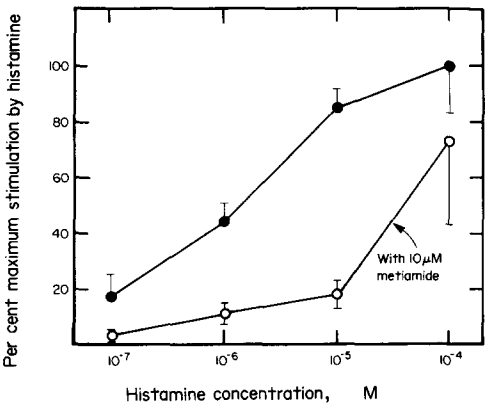

Fig. 4. Effects of various concentrations of histamine alone (●——●) or in combination with 10 μM metiamide (○——○) on cyclic AMP formation in intact cells. Maximum stimulation was considered to have been obtained at 0.1 mM histamine and is represented as 100 per cent. The enzyme activity in the presence of both a given concentration of histamine and of 10 μM metiamide is expressed as per cent maximum stimulation by histamine. The data for metiamide are mean values and ranges for at least four determinations. Metiamide at 10 μM alone did not appreciably alter basal cAMP levels. These data have been used to calculate K_i from the relationship $EC'_{50}/EC_{50} = 1 + [1]/K_i$, where EC'_{50} and EC_{50} are concentrations of histamine required to give half-maximal activation of the enzyme in the presence or absence of metiamide, respectively, and [1] is the concentration of metiamide used.

remained constant at 10 μM and the concentration of the antagonist was varied. At no concentration tested did any of the antagonists alter basal cAMP levels. Low concentrations of the H_2 antagonists metiamide and cimetidine, blocked the histamine-induced cyclase, with respective inhibition constants of 0.18 and 0.22 μM. In contrast, the H_1 antagonist pyrilamine or the classical serotonin antagonist methysergide had little inhibitory effect, with respective inhibition constants of more than 20 and 40 μM. In another set of experiments, the concentration of metiamide was held constant and the concentration of histamine was varied. In the presence of 10 μM metiamide, the EC_{50} for histamine increased from 1.4 to 38 μM (Fig. 4). The K_i was calculated to be 0.38 μM according to the dose–ratio method of analysis of the equilibrium dissociation constants [27].

The ability of various H_1 and H_2 specific agonists to stimulate this histamine sensitive adenylate cyclase was examined. Data in Fig. 5 demonstrate the higher potency in this system of the H_2 agonists dimaprit [28] and 4-methyl histamine when compared to the H_1 agonists 2-methyl histamine or the putative H_2 agonist clonidine. Whereas the H_2 agonists were able to exert the same maximal stimulation observed with histamine, 2-methyl histamine and clonidine induced only 40 per cent of this maximal response.

It would be expected that if these agonists were to exert their stimulatory effects via the histamine receptor then they should be antagonized by the same compounds that block the histamine response. Metiamide at 100 μM completely antagonized the stimulation due to dimaprit, 4-methyl histamine, and clonidine (agonist tested at 10 and 100 μM).

Impromidine, a recently described H_2 agonist, has been shown to be 900–4800 per cent more active than histamine at H_2 receptors of guinea pig atrium and rat uterus [29]. In the calf-aorta cell system, impromidine exerted its maximum stimulation at 1 μM; this effect, however, was only 32 per cent of the maximal stimulation obtainable with histamine. This stimulation by impromidine could be attributed to interaction with the histamine receptor since it could be completely blocked with 10 μM cimetidine. To examine further the action of impromidine at this histamine receptor, impromidine was added to cell plates just prior to the addition of 10 μM histamine. Figure 6 demonstrates impromidine's potent antagonism of the histamine response. Even without taking into account the slight stimulation by impromidine, the calculated K_i value (0.48 μM) for impromidine is in the range determined for both metiamide and cimetidine and is indicative of the

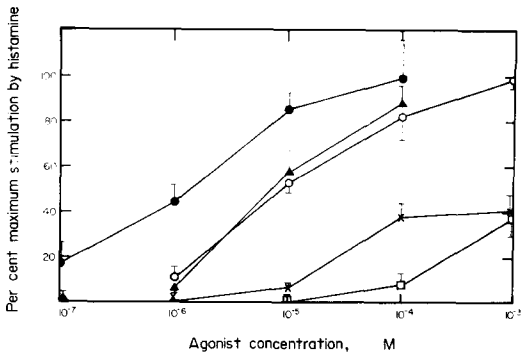

Fig. 5. Comparison of the stimulatory effects, on histamine stimulation, of various histamine agonists in intact cells. Maximum histamine stimulation was considered to have been obtained at 0.1 mM histamine and is represented as 100 per cent. Each value is an average of three experiments performed in triplicate. Key: histamine (●——●); 4-methyl histamine (○——○); 2-methyl histamine (X——X); dimaprit (▲——▲); and clonidine (□——□).

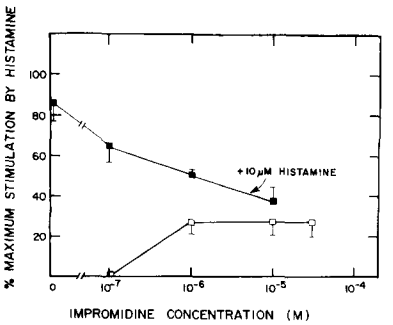

Fig. 6. Effects of various concentrations of impromidine alone (□——□) or in combination with 10 μM histamine (■——■) on cAMP formation in intact cells. Data are expressed as percentages of the maximal stimulation of cAMP formation produced by histamine (i.e. that obtained with 0.1 mM histamine). Histamine alone at 10 μM produced 85 per cent of maximal stimulation (see Fig. 4). Each value is an average of three experiments performed in triplicate.

strong blocking effect of impromidine at this histamine receptor. This is the first report of mixed agonist–antagonist properties of impromidine.

DISCUSSION

To our knowledge there have been only a few reports of serotonin receptors associated with an adenylate cyclase in cultured cells. Studies by Buonassi and Venter [11] indicated that serotonin leads to an increase in both cAMP and cGMP in cultured rabbit vascular endothelial cells, but the investigators did not report pharmacological characterization of the receptor. A recent investigation described the presence of two types of serotonin receptors in NCB-20 neuroblastoma–brain hybrid cells [10]. One type of receptor was found to be associated with an adenylate cyclase whereas the other appeared to mediate membrane depolarization and acetylcholine release. In homogenate preparations of the hybrid cell, serotonin, LSD and metergoline led to an elevation in cAMP levels. Both the serotonin and the LSD stimulation could be antagonized by mianserine and cyproheptadine, and LSD was found to be a mixed agonist–antagonist. The serotonin receptor present in the neuroblastoma–brain hybrid appears to resemble the serotonin receptors associated with adenylate cyclase found in mammalian brain. Brain studies have demonstrated the inhibitory effects of classical serotonin antagonists on stimulation of adenylate cyclase by serotonin in monkey anterior limbic cortex [30], rat colliculus and rat striatum [31]. However, these antagonists in general are not totally selective, for they also inhibit stimulation of adenylate cyclase by dopamine. The atypical neuroleptic drug, molindone, recently has been shown to exhibit selectivity for the brain adenylate cyclase stimulated by serotonin, as opposed to the adenylate cyclase stimulated by dopamine [32, 33]. The calf-aorta serotonin receptor appears to be quite different from the serotonin receptors associated with adenylate cyclase in brain or neuroblastoma–brain hybrid cells, as indicated in particular by the insensitivity of the calf-aorta receptors to LSD and molindone.

The serotonin receptors associated with adenylate cyclase of cultured calf-aorta cells and brain receptors may have in common some structural requirements for activation. Thus, in rat brain homogenates [31] as well as in the studies reported here, 5-methoxy and *N*-methyl analogues of serotonin retained activity. In contrast, several drugs which effectively antagonized mammalian brain serotonin-stimulated adenylate cyclase did not block the serotonin stimulation in these cell cultures. Further comparative studies will be required to better understand the similarities and differences among these various serotonin receptors.

Two types of histamine receptors have been defined on the basis of differences in response to antagonists [34]. Investigations carried out with tissues derived from the CNS as well as from other tissues or cell types have demonstrated coupling of both H_1 and H_2 histamine receptors to adenylate cyclase and in some instances to guanylate cyclase. Histamine has been shown to elevate cAMP in several regions of guinea pig brain via an H_2 receptor [35] and in cultured human astrocytoma cell lines (receptor type not identified) [8], and to increase cGMP in cultured mouse neuroblastoma via an H_1 receptor [9]. Histamine has been implicated in modulating immunological function of lymphocytes by activating H_2 receptors associated with adenylate cyclase [36]. Histamine has also been found to elevate cGMP levels in cultured rabbit endothelial cells, but the receptor type was not identified. In the present report, an exclusively H_2-type receptor located on cultured calf-aorta smooth muscle cells and associated solely with an adenylate cyclase has been demonstrated. Identification of an H_2 histamine receptor has depended generally on the ability of classical H_2 antagonists and the inability of the H_1 antagonist to block the stimulation of adenylate cyclase by histamine and H_2 agonists. The average inhibition constant of this histamine sensitive adenylate cyclase for metiamide based on the two methods of determination is 0.28 μM, which is similar to the K_i value of 0.22 μM for cimetidine calculated from data in Fig. 3. These values suggest a somewhat greater affinity of metiamide for the H_2 receptor in these cultures than has been reported for the H_2 receptors in guinea pig brain (K_i = 1.0 μM [21]), guinea pig ventricular muscle (K_i = 1.4 μM [22]), and rat uterus (K_i = 0.75 μM [37]), and perhaps are indicative of differences in these receptors.

The antagonism observed with impromidine suggests a re-examination of its stimulatory effects in other histamine adenylate cyclase systems and possible reclassification of this drug as a mixed H_2 agonist–antagonist. Structure modification of this diimidazole ring compound may lead to a powerful antagonist without agonist properties. In conclusion, the high sensitivity of the H_2-histamine adenylate cyclase of the cultured calf-aorta smooth muscle cell and the relative ease of measuring cAMP levels make this system ideal for screening putative H_2 antagonists and agonists.

Acknowledgements—We thank Dr. A. M. Adamany for providing the calf-aorta smooth muscle cell lines. This work was supported by NIH 5T 32 AG0052 and NIH 5PO1 AG 00374. The data in this paper are from a thesis to be submitted by A. R. L. in partial fulfillment for the Degree of Doctor of Philosophy in the Sue Golding Graduate Division of Medical Sciences, Albert Einstein College of Medicine, Yeshiva Universtiy.

REFERENCES

1. Z. S. Ercan and R. K. Turker, *Pharmacology* **15**, 118 (1977).
2. W. C. DeMello, *Eur. J. Pharmac,* **35**, 315 (1976).
3. P. D. I. Richardson and P. G. Withrington, *Br. J. Pharmac.* **60**, 123 (1977).
4. M. A. McGrath and J. T. Shepherd, *Fedn. Proc.* **37**, 195 (1978).
5. A. H. Soll and A. Wollin, *Gastroenterology* **72**, 1166 (1977).
6. C. Johnson and H. Mizoguchi, *J. Pharmac. exp. Ther.* **200**, 174 (1977).
7. S. Katsuki and F. Murad, *Molec. Pharmac.* **13**, 330 (1977).
8. R. B. Clark and J. P. Perkins, *Proc. natn. Acad. Sci. U.S.A.* **68**, 2757 (1971).
9. E. Richelson, *Science* **201**, 769 (1978).

10. J. MacDermont, H. Higashida, S. Wilson, H. Matsuzawa, J. Minna and M. Nirenberg, *Proc. natn. Acad. Sci. U.S.A.* **76**, 1135 (1979).
11. V. Buonassi and J. C. Venter, *Proc. natn. Acad. Sci. U.S.A.* **73**, 1612 (1976).
12. S. Fowler, H. Shio and H. Wolinsky, *J. Cell Biol.* **75**, 166 (1977).
13. B. Coltroff-Schiller, S. Goldfischer, A. M. Adamany and H. Wolinsky, *Am. J. Path.* **83**, 45 (1976).
14. J. T. Mills and A. M. Adamany, *J. biol. Chem.* **253**, 5270 (1978).
15. G. Bhargava, S. G. Horowitz, L. Rifas and M. H. Makman, *Fedn. Proc.* **37**, 3048 (1978).
16. G. Bhargava, L. Rifas and M. H. Makman, *J. cell. Physiol.* **100**, 365 (1979).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
18. A. G. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
19. Y. C. Cheng and W. H. Prusoff, *Biochem. Pharmac.* **22**, 3099 (1973).
20. M. H. Makman, in *Biochemical Actions of Hormones* (Ed. G. Litwack), Vol. 4, pp. 407–496. Academic Press, New York (1977).
21. P. Kanof and P. Greengard, *J. Pharmac exp. Ther.* **209**, 87 (1979).
22. P. Kanof and P. Greengard, *Molec. Pharmac.* **15**, 445 (1979).
23. H. S. Ahn and M. H. Makman, *Brian Res.* **162**, 77 (1979).
24. E. Apperly, P. P. A. Humphrey and G. P. Levy, *Br. J. Pharmac.* **58**, 211 (1976).
25. J. Daly, in *Handbook of Psychopharmacology* (Eds. L. T. Iversen, S. D. Iversen and S. H. Snyder), Vol. 5, pp. 47–139. Plenum Press, New York (1975).
26. J. W. Daly, M. Huang and H. Shimizu, *Adv. Cyclic Nucleotide Res.* **1**, 375 (1972).
27. D. R. Waud, *Pharmac. Rev.* **20**, 49 (1968).
28. M. Parsons, D. A. A. Owen, C. R. Ganellin and G. J. Durant, *Agents Actions* **7**, 31 (1977).
29. G. J. Durant, W. A. M. Duncan, C. R. Ganellin, M. E. Parsons, R. C. Blackmore and A. C. Rasmussen, *Nature, Lond.* **276**, 403 (1978).
30. H. S. Ahn and M. H. Makman, *Brain Res.* **153**, 636 (1978).
31. A. Enjalbert, S. Bourgoin, M. Hamon, J. Adrien and J. Bockaert, *Molec. Pharmac.* **14**, (1978).
32. H. S. Ahn and M. H. Makman, *Life Sci.* **23**, 507 (1978).
33. M. R. Rosenfeld, M. H. Makman, H. S. Ahn, L. J. Thal, R. K. Mishra and R. Katzman, in *Ergot Compounds and Brain Function—Neuroendocrine and Neuropsychiatric Aspects* (Eds. M. Goldstein, D. D. Calne, A. Lieberman and M. O. Thorner), pp. 83–94. Raven Press, New York (1980).
34. J. W. Black, W. A. M. Duncan, C. J. Durant, C. R. Ganellin and E. M. Parsons, *Nature, Lond.* **236**, 385 (1972).
35. L. R. Hegstrand, P. D. Kanof and P. Greengard, *Nature Lond.* **260**, 163 (1976).
36. W. Roszkowski, M. Plaut and L. M. Lichtenstein, *Science* **195**, 683 (1977).
37. J. W. Black, G. J. Durant, J. C. Emmett and C. R. Ganellin, *Nature, Lond.* **248**, 65 (1974).