Biochemical Pharmacology, Vol. 30, No. 15, pp. 2182–2185, 1981.
Printed in Great Britain.

0006-2952/81/152182-04 $02.00/0

Antidepressant inhibition of H_1- and H_2-histamine-receptor-mediated adenylate cyclase in [2-^{3}H]adenine-prelabeled vesicular preparations from guinea pig brain

(*Received* 29 *October* 1980; *accepted* 29 *January* 1981)

An antihistaminic mechanism of action involving H_2-receptors has been proposed to explain some of the pharmacological effects of antidepressants [1], including their activity as therapeutic agents [2]. Antidepressants such as amitriptyline and imipramine inhibit histamine activation of adenylate cyclase in homogenates of guinea pig neocortex or hippocampus [1, 2] that had apparently lost H_1- but had retained H_2-receptor-linked adenylate cyclase activity [3]. The ability of antidepressants to act as H_2-receptor antagonists was considered by Kanof and Greengard [2] to be consistent with the ability of the H_2-receptor antagonist metiamide to prevent the depressant effect of histamine on neuronal firing frequency in cerebral cortex [4].

Histamine-induced depression of cortical neurones, however, was shown to involve H_1- as well as H_2-receptors, and the H_1-antagonist mepyramine antagonized the depressant effects of histamine and of the H_1-agonist 2-methylhistamine [5]. Other H_1-antagonists such as tripelennamine and chlorcyclizine were also able to abolish the depressant effects of histamine on neuronal firing frequency in cerebral cortex [6]. Moreover, tricyclic antidepressants were able to block H_1-receptor-mediated cyclic GMP synthesis in mouse neuroblastoma cells [7] and to block the binding of [^{3}H]mepyramine to H_1-receptors in mammalian brain membranes [8].

In a previous study utilizing vesicular preparations of cerebral cortex which retained both H_1- and H_2-receptor-linked adenylate cyclase, the tetracyclic antidepressant maprotiline was found to be primarily an H_1-receptor antagonist [9]. Thus, in the present study, other antidepressants were also tested for the ability to inhibit activation of adenylate cyclase via H_1- or H_2-receptors. [2-^{3}H]Adenine-prelabeled vesicles prepared from guinea pig cerebral cortex and stimulated by the H_1-receptor agonist 2-methylhistamine provided on H_1-receptor test system; vesicles from guinea pig hippocampus, stimulated with the H_2-receptor agonist 4-methylhistamine, provided an H_2-receptor test system; the results indicate that antidepressants were more potent as H_1-receptor antagonists.

Male guinea pigs (Hartley strain, Marland Breeding Farms, Hewitt, NJ), weighing 300–400 g and fed Purina Laboratory chow *ad lib.* were used in all experiments. Dowex 50 W-X8 cation exchange resin, 100–200 mesh, was purchased from Bio-Rad Laboratories (Rockville Center, NY); histamine from the Aldrich Chemical Co. (Metuchen, NJ); and [2-^{3}H]adenine (15–30 Ci/mmole, 1 mCi/ml) and [U-^{3}H]cyclic AMP (30–50 Ci/mmole) from the New England Nuclear Corp. (Boston MA). Dimethindene, maprotiline, and tripelennamine were products of the Ciba-Geigy Corp. (Summit, NJ). Fluoxetine, 2-methylhistamine and mianserin were prepared by Mr. R. Dziemian, and promethazine was prepared by Dr. R. Emmons and Dr. J. Nelson of these laboratories. Amitriptyline and cyproheptadine were obtained from Merck & Co. Inc. (Rahway, NJ); chlorpromazine, cimetidine, metiamide and 4-methylhistamine from Smith Kline & French (Philadelphia, PA); ketotifen from Sandoz, Inc. (East Hanover, NJ); haloperidol from McNeil Laboratory, Inc. (Fort Washington, PA); trimipramine from Ives Laboratories, Inc. (New York, NY); and fluphenazine from E. R. Squibb & & Sons Inc. (Princeton, NJ). Plastic tubes and pipettes were from Falcon (Cockeysville, MD); glass homogenizers from Kontes (Vineland, NJ); and glass scintillation vials from Kimble (Toledo, OH).

The basic procedure of Chasin *et al.* [10] as previously modified [9], was used for the preparation and labeling of brain vesicles. An even simpler method was used in some of the experiments as follows. Guinea pigs were decapitated and the brains were quickly removed and surrounded with ice. The entire cerebral cortex including the hippocampus from one brain, or the hippocampus alone from four brains, was quickly dissected on ice, cut into a few pieces, and partially homogenized with a single downward stroke in an all-glass, motor driven 15-ml Tenbroeck homogenizer (Kontes, K-885000) with 12 ml of ice-cold Krebs–Ringer bicarbonate buffer, pH 7.4, equilibrated with O_2–CO_2 (95%–5%) and containing 10 mM glucose and 10 μM EDTA. The pestle was removed by hand and the remaining tissue plug in the bottom of the tube was dislodged by a spatula. Homogenization was continued with one to three brief additional downward strokes of the motor-driven pestle (the minimum number of strokes necessary for complete disruption of the initial tissue mass). After each stroke, the motor was shut off and the tube was then returned by hand to its original starting position. The homogenate was added to a 50-ml plastic tube (Falcon, No. 2070), brought to a volume of 40 ml by the addition of cold buffer, and centrifuged at 1000 *g* for 15 min at 1–2°. The supernatant fraction was discarded, and the sediment was resuspended in 8 ml of cold buffer by pulling it into, and discharging it from, a 10-ml plastic pipette (Falcon, No. 7530) at least twice. Adenine (10 μl of a solution containing 0.11 mg/ml) and [2-^{3}H] adenine (60 μl of a solution containing 1 mCi/ml) were added, and the tube was gassed with O_2–CO_2, capped, and incubated at 37° with shaking for 20 min. Cold buffer was added to bring the contents of the tube to 40 ml and it was centrifuged under the same conditions as above. The supernatant fracture was discarded and the sediment was resuspended in 8 ml of cold buffer in the manner described above and brought to 40 ml with cold buffer. Aliquots (1 ml) of brain vesicles were added to glass liquid-scintillation vials (Kimble, No. 74500–20) and preincubated with shaking (104 cycles/min) at 37° for 40 min under O_2–CO_2.

Following the preincubation period, 0.5-ml aliquots of solutions containing buffer plus 2-methylhistamine (H_1-agonist) and test compounds, at three times the final desired concentration, were added to the 1-ml aliquots of vesicles from cerebral cortex, and incubation was continued with shaking at 37° under O_2–CO_2 for 15 min (H_1-receptor adenylate cyclase system). Each vial then received 0.5 ml of trichloroacetic acid (240 g/l) containing cyclic AMP (20 mg/l). Following centrifugation for 7 min at 1000 *g* in 15-ml centrifuge tubes (Falcon, No. 2087), the supernatant fractions were assayed for [^{3}H]cyclic AMP. During the incubation period (above), the final concentration of 2-methylhistamine was 100 μM. Each antagonist was tested at three or more concentrations, and each concentration was tested in three or four replications, to establish the concentration required for 50 per cent inhibition (IC_{50} value); antagonist potency was confirmed in separate

experiments. The H_2-receptor adenylate cyclase system was as described for the H_1-receptor system except that vesicles were prepared from hippocampus and incubated with 4-methylhistamine (H_2-agonist) at a final concentration of 100 μM. The assay for [^{3}H]cyclic AMP was essentially that of Krishna *et al.* [11] as described previously [9].

Concentration–activity curves for histamine and 2-methylhistamine with [2-^{3}H]adenine-prelabeled vesicles from guinea pig cerebral cortex and for 4-methylhistamine with vesicles from hippocampus are shown in Figs. 1 and 2. Although 2-methylhistamine may not have been entirely selective as an H_1-histamine receptor agonist [12], it was used at 100 μM for subsequent testing so that its activity was well below maximum and only a small fraction of the activity obtained with histamine (Fig. 1). 4-Methylhistamine was more selective as an H_2-histamine receptor agonist but nevertheless, when used at 100 μM, its activity was well below that with histamine (Figs. 1 and 2). The use of the mixed agonist histamine with the cortical vesicle preparation exhibited activity that was more characteristic of H_1-receptors (Table 1), possibly because of an abundance of such receptors in this tissue. Vesicles prepared from cerebral cortex and stimulated by 2-methylhistamine were highly sensitive to standard H_1-receptor antagonists, with IC_{50} values ranging from 0.005 to 0.06 μM (Table 1). When tested on the H_2-receptor system composed of hippocampal vesicles and 4-methylhistamine, the H_1-antagonists were 81 to 2600 times less potent.

The H_2-receptor antagonist metiamide had been shown previously to be effective against selective H_1-receptor agonists in tissue slice [15] or vesicular [9] preparations of brain tissue, in contrast to the low potency of cimetidine, the metiamide analog, in a peripheral H_1-receptor system such as guinea pig ileum [16]. In the present study, cimetidine and metiamide were active as antagonists in the H_1-system as well as in the H_2-system (Table 1). The ability of H_2-antagonists to inhibit H_1-receptor activated adenylate cyclase is consistent with the postulated role of H_2-receptors in coupling H_1-receptors with adenylate cyclase [17, 18]. Some of the antidepressants studied—amitriptyline, trimipramine, mianserin, and maprotiline—were highly potent in the H_1-system; potencies in the H_2-system were 28, 49, 76 and 407 times less respectively (Table 1). Imipramine was less active than other antidepressants in the H_1-system but its potency was relatively high in the H_2-system. For example, imipramine was five times less potent

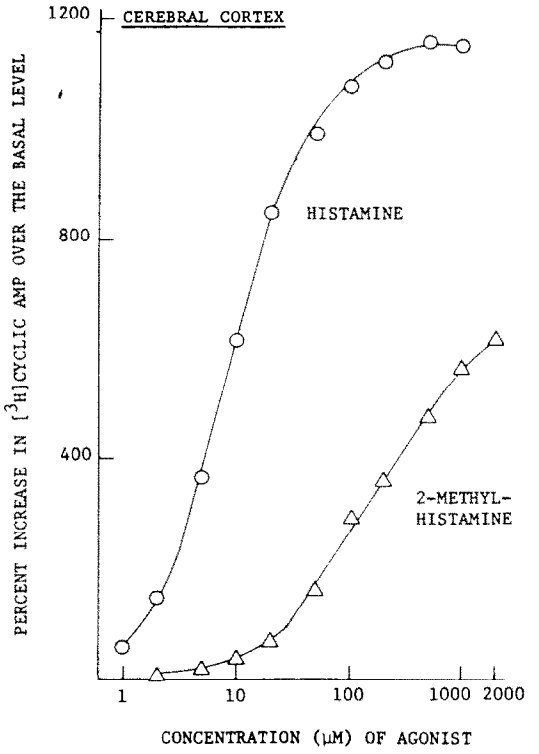

Fig. 1. Adenylate cyclase activity of [2-^{3}H]adenine-prelabeled vesicles from guinea pig cerebral cortex stimulated by histamine or 2-methylhistamine. Forty milliliters of vesicles were prepared from the cerebral cortex of one guinea pig. Following labeling with [2-^{3}H]adenine and preincubation, 1 ml aliquots were incubated in the presence of various concentrations of histamine or 2-methylhistamine for an additional 15 min, in a total volume of 1.5 ml. The basal [^{3}H]cyclic AMP level in the histamine experiment was 700 cpm; maximum [^{3}H]cyclic AMP formation in the presence of histamine was 8200 cpm, representing a 6.7 per cent conversion of [2-^{3}H]adenine (taken up by the vesicles) into [^{3}H]cyclic AMP when the increase in [^{3}H]cyclic AMP was corrected for aliquot and recovery factors.
In the 2-methylhistamine experiment, the basal [^{3}H]cyclic AMP level was 423 cpm and activity at the highest concentration of 2-methylhistamine used was 2622 cpm, representing a 2.4 per cent conversion of [2-^{3}H]adenine into [^{3}H]cyclic AMP when the increase in the [^{3}H]cyclic AMP cpm was corrected for aliquot and recovery factors. Two separate experiments are shown, and each point on both curves is the mean of triplicate or quadruplicate replications.

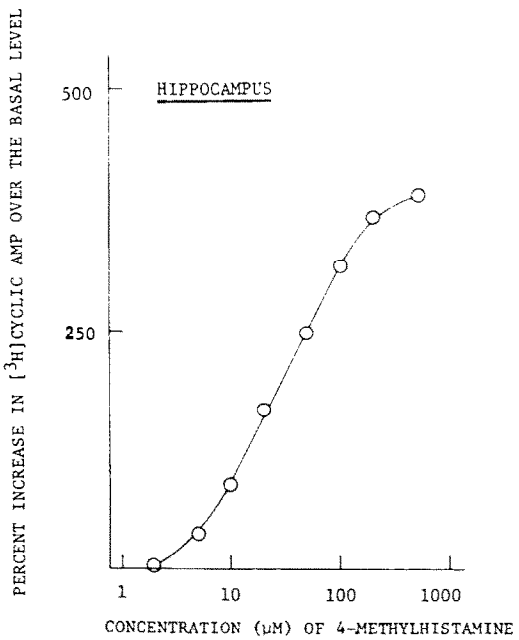

Fig. 2. Adenylate cyclase activity of [2-^{3}H]adenine-prelabeled vesicles from guinea pig hippocampus stimulated by 4-methylhistamine. Experimental conditions are described in the legend of Fig. 1 except that vesicles were prepared from the hippocampal tissue of four guinea pigs, and 4-methylhistamine was used as the agonist. The basal [^{3}H]cyclic AMP level was 347 cpm; maximum [^{3}H]cyclic AMP formation at 500 μM 4-methylhistamine was 1361 cpm, representing a 1.55 per cent conversion of [2-^{3}H]adenine (taken up by the vesicles) into [^{3}H]cyclic AMP when [^{3}H]cyclic AMP was corrected for aliquot and recovery factors. Each point is the mean of triplicate or quadruplicate replications in a single experiment.

Table 1. Effects of various drugs on histamine-receptor-linked adenylate cyclase in guinea pig brain

Drugs	IC_{50} (μM) [2-^{3}H]Adenine-prelabeled vesicles (a) Cortex plus histamine (50 μM)	(a) Cortex plus 2-methylhistamine (100 μM)	(b) Hippocampus plus 4-methylhistamine (100 μM)	Ratio b/a	Homogenate + ATP* Hippocampus plus histamine (100 μM)
H_1-Receptor antagonists					
Dimethindene	0.012	0.005	13	2600	
Ketotifen	0·016	0·006	13	2167	
Cyproheptadine	0.024	0.013	1.1	84	
Promethazine	0.047	0.038	3.1	81	0.4 [13]
Tripelennamine	0.32	0.060	100	1666	20 [13]
H_2-Receptor antagonists					
Cimetidine	5.8	1.2	1.6	1.3	4† [14], 12 [13]
Metiamide	8.5	1.9	3.0	1.6	10† [3], 12 [13]
Antidepressants					
Amitriptyline	0.048	0.035	1.0	28	0.66 [2]
Trimipramine	0.083	0.086	4.2	49	
Mianserin	0.19	0.05	3.8	76	0.88 [2]
Maprotiline	0.50	0.14	57	407	
Imipramine	1.9	0.48	4.5	9	1.9 [2]
Fluoxetine	>100	>10	>100		21 [2]

* Data were taken from the literature. References are shown in brackets.

† Cimetidine at 4 and 10 μM caused approximately 44 and 66 percent inhibition, respectively (Fig. 1, Ref. 14). Metiamide at 10 μM inhibited the increase in cyclic AMP due to histamine by approximately 45 percent (Fig. 1, Ref. 3).

than trimipramine in the H_1-system but was approximately equipotent with trimipramine in the H_2-system. Some selective serotonin reuptake inhibitors such as fluoxetine were reported to be much weaker antagonists than other antidepressants in the homogenate H_2-system [2]. In both the H_1- and the H_2-systems, fluoxetine was relatively inactive.

The two laboratories that drew attention to the possibility that antidepressants act pharmacologically by antagonizing H_2-histamine receptors in brain [1, 2] used a membrane-type homogenate system that contained adenylate cyclase and the H_2-receptor but which apparently did not retain sufficient structural elements, so that cofactor GTP and substrate ATP had to be added. H_1-receptor-linked adenylate cyclase could not be demonstrated, possibly because of the liability or inactivation of H_1-receptors in this system [3]. There is, therefore, no evidence, using a membrane-type homogenate preparation that H_1-receptors are directly linked to adenylate cyclase. In tissue slices or vesicular systems, which have provided evidence for the association of H_1-receptors with adenylate cyclase, H_1-receptors may be indirectly linked to adenylate cyclase or dependent on the interaction of other receptors. For example, it has been suggested that the prior H_2-receptor stimulation is necessary for H_1-receptor interaction with adenylate cyclase [18] and that H_2- and adenosine-receptors mediate the action of H_1-receptors on adenylate cyclase [17].

To demonstrate that antidepressants might exert their antihistamine effects via H_1-receptors, it was considered necessary to provide further evidence for the association of H_1-receptors with adenylate cyclase, whether or not such an association was direct or indirect. Tissue slice studies had been the first to show that H_1-receptors might be associated with adenylate cyclase in brain [15, 19], and a recent study indicated that the H_1-antagonist mepyramine at 0.005 μM inhibited histamine activated adenylate cyclase in hippocampal slices [18]. Vesicular preparations consisting of membrane-enclosed portions of nerve cells, which functioned like tissue slices in that they used endogenous ATP as substrate and did not require the addition of GTP, also provided evidence of H_1-receptor-associated adenylate cyclase [9]. The present study using vesicular preparations of guinea pig cortex shows that five standard H_1-receptor antagonists inhibited the action of the H_1-agonist 2-methylhistamine, with IC_{50} values of 0.005 to 0.06 μM. The high sensitivity of the H_1-receptor system to these antagonists is consistent with a selective action of 2-methylhistamine of H_1-receptors in the present study and contrasts with the reported non-selectivity of 2-methylhistamine in a system believed to be devoid of H_1-receptors [3]. In that study even a relatively high concentration (1 μM) of an H_1-antagonist was unable to oppose the action of histamine. The similarly high sensitivity to these antagonists of adenylate cyclase stimulated by an $H_1 + H_2$-agonist (histamine) and an H_1-agonist (2-methylhistamine) (Table 1) supports the view that there is a preponderance of H_1-receptors in guinea pig neocortex [15].

Further evidence for H_1-receptor-associated adenylate cyclase in vesicular preparations of brain is provided by H_1-receptor binding studies which have, in general, the same degree of sensitivity to H_1-antagonists. For example, the affinity constants for promethazine, chlorpromazine, fluphenazine, and haloperidol obtained by the displacement of the H_1-receptor ligand [^{3}H]mepyramine from a prep-

aration of guinea pig brain were 70, 43, 15, and 7300 nM respectively [20]; approximate inhibition constants for these compounds in the cortical H_1-system were 26, 120, 66 and 1500 nM respectively (S. Psychoyos, unpublished data).

Recent pharmacological studies have demonstrated the efficacy of the antidepressant amitriptyline as both an H_1- and H_2-antagonist. It was among the most potent H_1-antagonists known in its ability to block histamine-induced contractions of guinea pig ileum [21], and *in vivo* attenuated dimaprit-induced hypothermia in rats to the same extent as the intraventricularly administered H_2-antagonist cimetidine [22]. In addition, classical H_1-antagonists such as tripelennamine and chlorpheniramine were found to be active in a pharmacological test procedure for antidepressants [23]. The use of the vesicular adenylate cyclase methodology thus draws attention to the H_1-antagonist efficacy of amitriptyline and other antidepressants in brain tissue and, also, to their lesser efficacy as H_2-antagonists.

In summary, the effects of antidepressants and histamine antagonists on the activation of cyclic AMP systems in [2-^{3}H]adenine-prelabeled vesicles of guinea pig cerebral cortex by 2-methylhistamine, an H_1-agonist, and on the activation of cyclic AMP systems in vesicles from hippocampus by 4-methylhistamine, an H_2-agonist, have been investigated. Further evidence that H_1-histamine receptors are associated with adenylate cyclase in brain has been provided. Antidepressants were more potent as inhibitors of adenylate cyclase activated via H_1-receptors than by acting directly on H_2-receptors.

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