JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1994, 45, 3, 419—428

J. BUGAJSKI, A. GĄDEK-MICHALSKA, J. BORYCZ, A. J. BUGAJSKI, R. GŁÓD

HISTAMINERGIC COMPONENTS IN CARBACHOL-INDUCED PITUITARY-ADRENOCORTICAL ACTIVITY

Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

The involvement of central histaminergic mechanisms in stimulation of the hypothalamic-pituitary-adrenal (HPA) axis by carbachol, a muscarinic cholinergic agonist, was investigated in conscious rats. The HPA activity was assessed indirectly, through corticosterone secretion. Carbachol given intracerebroventricularly elicited a dose-related increase in serum corticosterone levels. The corticosterone response to carbachol was totally abolished by systemic pretreatment 2 h earlier with α-fluoro-methylhistidine (α-FMH), a specific inhibitor of brain histamine synthesis, which also significantly decreased histamine level in hypothalamus. Mepyramine, a histamine H_1-receptor antagonist, moderately diminished the carbachol-induced corticosterone response and abolished the rise in hypothalamic histamine levels. Ranitidine, a H_2-receptor antagonist, considerably diminished the corticosterone response to carbachol but did not change the elevated hypothalamic histamine levels. Also atropine, a cholinergic antagonist, abolished the corticosterone response to carbachol, but did not significantly affect the carbachol-induced increase in hypothalamic histamine concentration. Ranitidine and atropine can directly block homologous hypothalamic receptors involved in CRF secretion. Partial inhibition of the carbachol-induced corticosterone secretion by mepyramine may be connected with prevention of the carbachol-induced increase in hypothalamic histamine content.

These results suggest that hypothalamic histamine and histamine receptors are involved in the HPA stimulation by the muscarinic agonist carbachol.

K e y w o r d s: *pituitary-adrenal axis, corticosterone, carbachol, hypothalamic histamine, brain histamine receptors.*

INTRODUCTION

Considerable evidence indicates that cholinergic neurons modulate the HPA axis, predominantly via stimulation of the hypothalamic release of CRF. Acetylcholine (ACh) and other cholinergic agonists are considered to stimulate the HPA axis (1—4). The muscarinic cholinergic agonist arecoline stimulates the PHA axis centrally, mainly via release of the endogenous CRF (1). Choline acetyltransferase (ChAT) neurons have been identified in the rat and human

hypothalamus. Also biochemical and pharmacological studies clearly indicate the presence of intrinsic cholinergic neurons in the hypothalamus (5). Several studies have demonstrated that ACh induces the release of CRF from the rat and sheep hypothalamus (3), but the response of an isolated tissue in vitro may not be representative of the normal physiological functioning. The anterior pituitary has no cholinergic nerve supply, nor does it contain the ACh forming enzyme choline transferase (6) but muscarine cholinergic receptors have been identified in the anterior and posterior pituitary (7). ACh appears in anterior pituitary, apparently as a humoral factor (8). Plasma corticosterone correlated positively with ACh levels in the cortex and striatum in physostigmine-treated rats (2). Evidently the CNS mechanism by which ACh can affect the HPA activity remains unclear and requires further investigation.

The participation of histamine and its receptors in the central stimulation of the HPA axis induced by cholinergic agonists is unclear. Our preliminary experiment suggested that the level of neuronal histamine is essential for the carbachol-induced stimulation of the HPA axis (9). Brain histamine and central histamine receptors are also significant for the expression of the stimulatory effects of clonidine (10), an α_2-adrenergic agonist, and β-endorphin (11) as well as opioids (12) on the secretion of corticosterone. In those experiments a significant depression of the neuronal histamine synthesis by α-FMH, a specific inhibitor of histidine decarboxylase (13, 14), diminished the HPA responses elicited by the opioids, clonidine and carbachol given intracerebroventricularly (icv). Histamine seems to be indispensable to the normal functioning of the corticotropinergic system (15—17). Also the histamine release, synthesis and turnover in the brain is regulated by muscarinic receptors (18, 19). In addition, histamine immunoreactive neuronal fibres and terminals are most numerous in the different hypothalamic nuclei (20).

Hence in the present study we attempted to find out whether, and to what extent central histamine receptors and hypothalamic histamine participate in the carbachol-induced HPA response.

MATERIALS AND METHODS

The experiments were performed on male Wistar rats weighing 200—230 g. The animals were housed in groups of 7 animals to a cage, for at least one week, on a diurnal light cycle at an ambient room temperature of 21° C. Water and standard laboratory food were available ad libitum. Drugs were injected in a volume of 10 µl, into the right lateral cerebral ventricle, using a Hamilton microsyringe, to rats whose skulls were prepared 24 h earlier, under light ether anesthesia, for free-hand icv injections (15). After injections of the drugs, the rats were returned to their cages and decapitated 1 h later. Control animals received simultaneously 10 µl of saline and

were left undisturbed until decapitation, concurrently with the animals injected with the drugs. Alpha-fluoromethylhistidine was injected intraperitoneally 2 h before icv administration of carbachol, i.e. 3 h before decapitation. Atropine and the histamine receptor antagonists mepyramine and ranitidine were administered 15 min before carbachol, given by the same route. All the experiments were performed between 9—11 h, and all decapitations took place between 11—12 h to avoid corticosterone fluctuations due to the diurnal rhythm.

Immediately after removal from the cage, the rats were decapitated and their trunk blood was collected and refrigerated. Within 2 h, the blood was centrifuged and the serum was removed and frozen at −80° C. The concentration of corticosterone was measured fluorometrically (21). For histamine determinations the rats were decapitated at the required time, their brains were quickly removed, placed on glas plates kept on ice and washed with ice-cold saline. The cerebella were discarded and the hypothalami were isolated and stored at −80° C until use. For determination of the histamine concentration a 10 or 20% (w/v) homogenate of the tissue was made in 0.4 M perchloric acid. The homogenate was centrifuged and supernatant was adjusted to pH 5—6 with 0.2 M KOH. Isolation and analysis of histamine was then carried out by modification of the procedure described by Kremzner and Pfeiffer (22). A 0.5 ml aliquot was passed through a Cellex P column (5 × 30 mm) and washed sequentially with 0.5 ml of 0.03 and 0.1 M sodium phosphate buffer (pH 6.2). Histamine was eluted with 1.5 ml of 0.07 M hydrochloric acid and, after condensation with O-phtaldialdehyde, it was estimated fluorometrically at 360/450 nm (23).

The first experimental series was performed to examine dose-related effects of carbachol on the HPA axis. Different doses of atropine were given to another group of rats 15 min prior to carbachol, to determine to what extent the stimulatory effect of carbachol on the HPA axis was mediated by muscarinic cholinergic receptors. In the second series of experiments we evaluated the effects of the α-FMH and histamine H_1 and H_2-receptor antagonists mepyramine and ranitidine on the carbachol-induced effects on corticosterone secretion. The rats were pretreated ip with α-FMH or icv with either antagonist 2 h or 15 min before carbachol, respectively. Hypothalamic histamine levels were assessed following intraventricular carbachol administration, and were correlated with changes in the HPA activity measured by serum corticosterone levels. The hypothalamic histamine levels were also measured in rats pretreated with atropine or histamine antagonists before carbachol, to compare the effects of those antagonists on serum corticosterone and hypothalamic histamine levels.

RESULTS

Effect of carbachol and atropine on serum corticosterone levels

Carbachol (0.1—10 μg), a cholinergic muscarinic receptor agonist, given icv induced a dose-related increase in serum corticosterone levels measured 1 h after administration (*Table 1*). Atropine, a cholinergic receptor antagonist, given alone icv in doses of 0.001—0.1 μg, did not substantially change the resting serum corticosterone levels. When given 15 min prior to carbachol, atropine (0.1 μg) abolished a typical rise in the serum corticosterone level elicited by carbachol (*Fig. 1*). This finding suggests that carbachol activates the pituitary-adrenocortical axis by a fairly selective stimulation of central muscarinic receptors.

Table I. Effect of carbachol on serum corticosterone and hypothalamic histamine levels

Treatment		Corticosterone µg/dl	Histamine ng/g tissue
Saline control	10 µl	5.2±0.8	400±17
Carbachol	1 µg	14.0±1.1^{+}	455±33
Saline control	10 µl	7.8±0.8	516±24
Carbachol	2 µg	33.2±6.1^{++}	642±88^{+}

Carbachol was given icv 1 h before decapitation of the rats. Values represent the mean ±SEM of 7 animals. $^{+}$ $p < 0.05$ and $^{++}$ $p < 0.001$ vs. saline control.

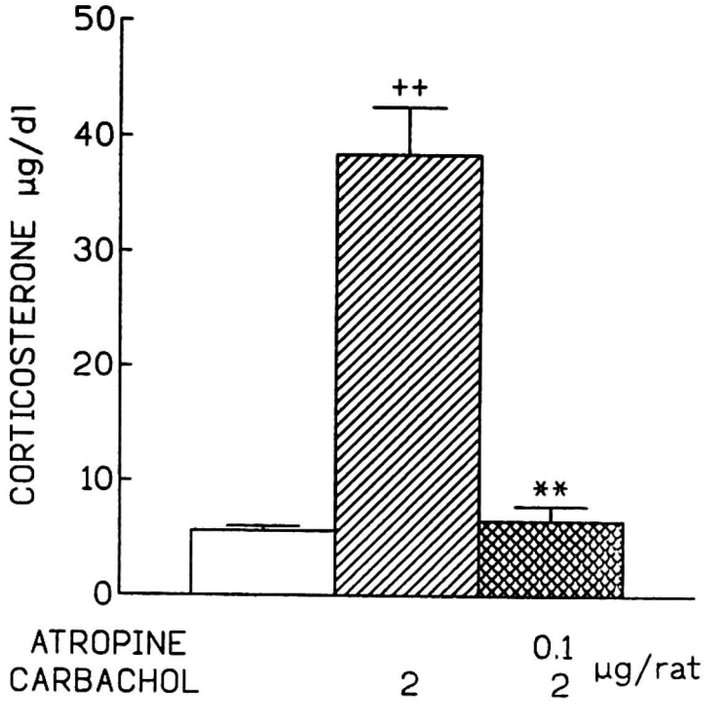

Fig. 1. Effect of atropine on carbachol-induced serum corticosterone concentration. The drugs were given icv, atropine 15 min prior to carbachol. In Fig. 1—6 the rats were decapitated 1hafter carbachol administration. Values represent the mean ±SEM of 7 rats. $^{++}$ $p < 0.001$ vs. saline controls and ** $p < 0.001$ vs. carbachol treated group.

Reduction of the hypothalamic histamine and carbachol-induced corticosterone response by α-FMH

Systemic administration of α-FMH (20 mg/kg), a brain histamine synthesis inhibitor, considerably decreased both the hypothalamic and whole brain histamine levels, measured 2 h later, but did not change the basal serum corticosterone level (10). α-FMH administered ip 2 h before carbachol almost totally abolished the increase in corticosterone secretion induced by that agonist in control saline pretreated rats (*Fig. 2*).

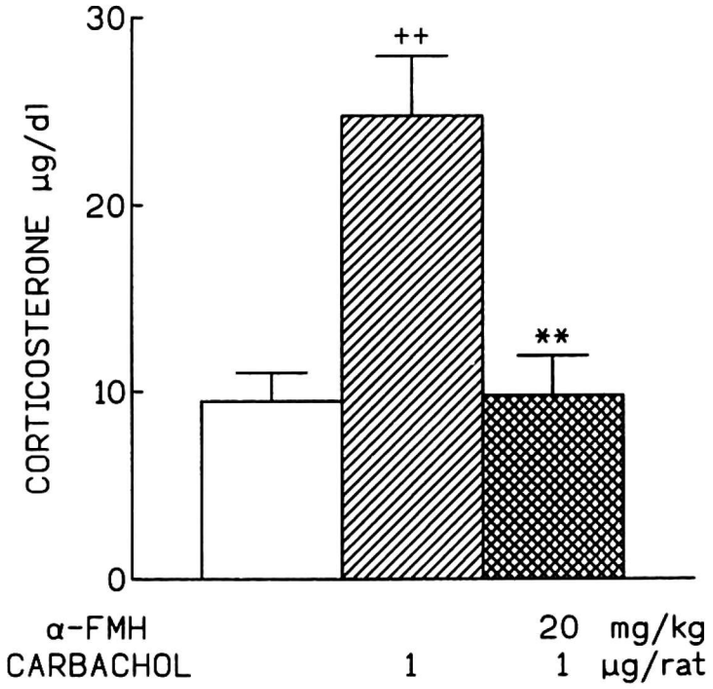

Fig. 2. Effect of α-FMH on carbachol-induced corticosterone response. α-FMH was given ip 2 h before icv carbachol. $^{++}$ $p < 0.001$ vs. saline controls and ** $p < 0.001$ vs. carbachol-treated group.

Effect of histamine blockers on the carbachol-induced corticosterone response

Pretreatment of rats with mepyramine (0.1 µg icv) markedly, but not significantly, diminished - by 30% - the rise in the serum corticosterone level elicited by carbachol. Ranitidine, a potent histamine H_2-receptor antagonist, diminished highly significantly - by 57% - the corticosterone response to carbachol (*Fig. 3*). Intraventricular injections of mepyramine and ranitidine in the absence of carbachol did not affect the serum corticosterone levels.

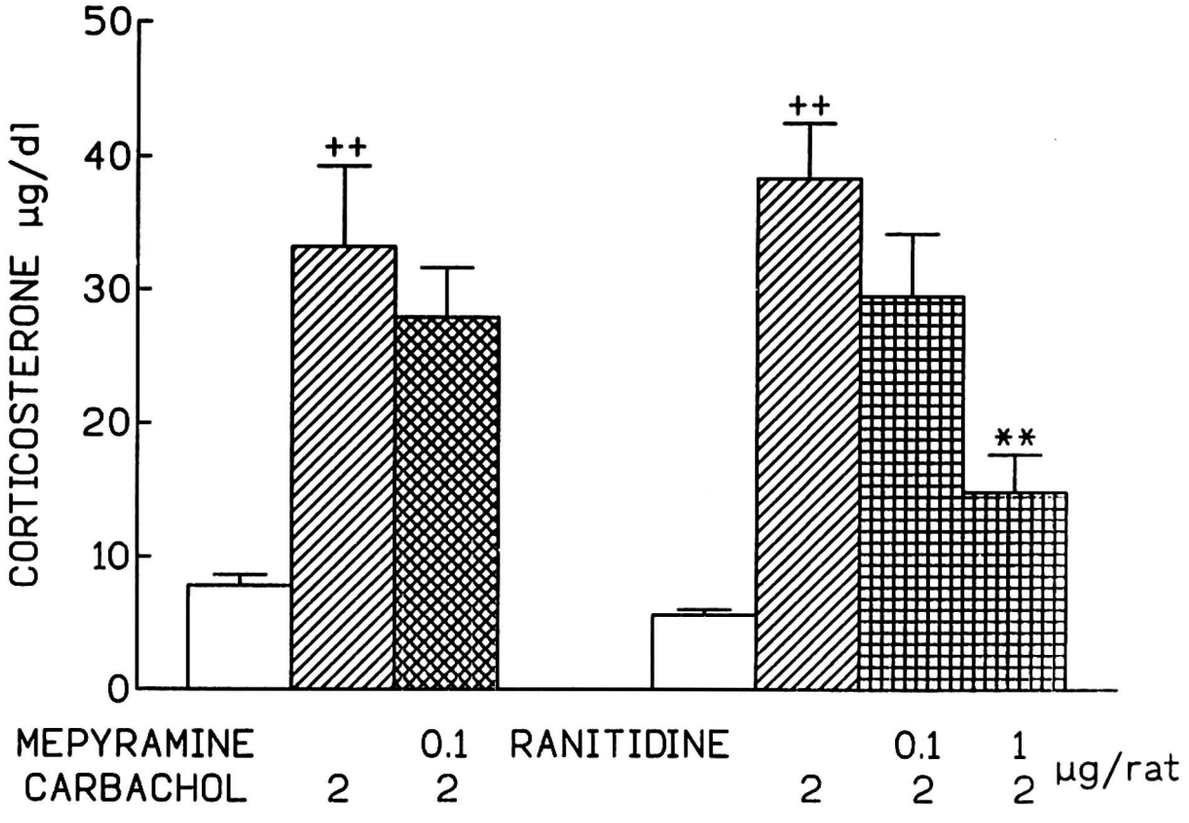

Fig. 3. Effect of mepyramine and ranitidine on carbachol-induced corticosterone response. The drugs were given icv, the antagonists 15 min before carbachol. $^{++}$ $p < 0.001$ vs. saline controls and ** $p < 0.001$ vs. carbachol treated group.

Changes in hypothalamic histamine levels

Carbachol given icv increased the serum corticosterone and also hypothalamic histamine levels. When used in doses of 1 and 2 μg, carbachol significantly raised the serum corticosterone level, by 169 and 434%, and

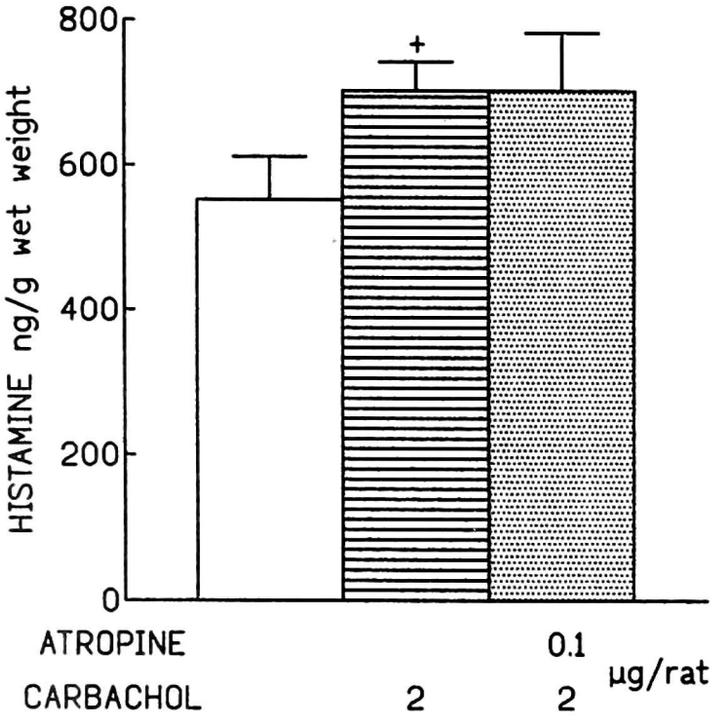

Fig. 4. Hypothalamic histamine content after icv carbachol and atropine given 15 min before carbachol. $^{+}$ $p < 0.05$ vs. saline controls.

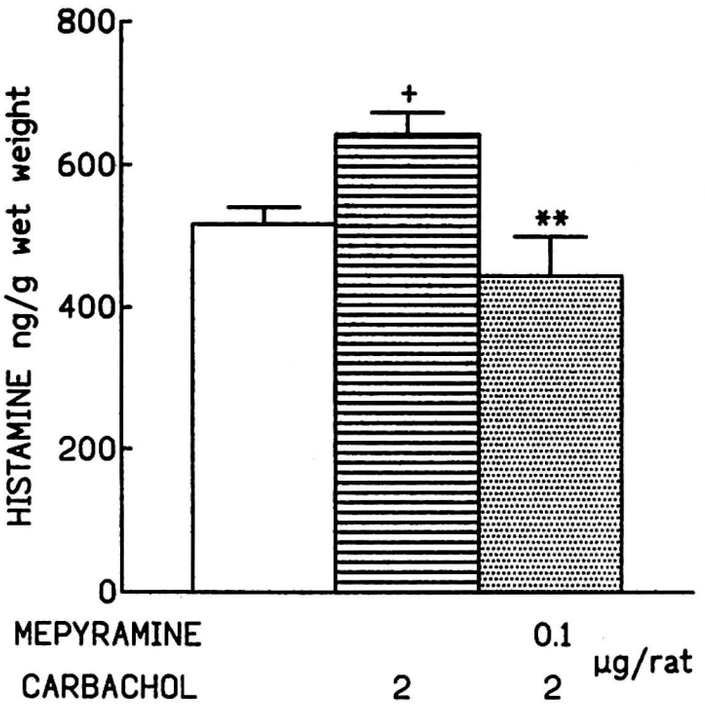

Fig. 5. Hypothalamic histamine content after icv carbachol and mepyramine given 15 min before carbachol. $^{+}$ $p < 0.05$ vs. saline controls and ** $p < 0.001$ vs. carbachol-treated group.

markedly increased the hypothalamic histamine content, by 14 and 24% ($p < 0.05$), respectively (*Table 1*). Neither atropine nor ranitidine exerted any significant effect on the carbachol-induced rise in the hypothalamic histamine level, but they reduced the carbachol-elicited increase in the serum cirticosterone (*Figs 4, 6*). On the other hand, mepyramine (0.1 μg) in a dose

which inhibited most effectively the carbachol-induced increase in the corticosterone secretion, considerably decreased the hypothalamic histamine level, even below the control level in saline-treated rats (*Fig. 5*). This result suggests that the antagonistic effect of atropine and ranitidine on the

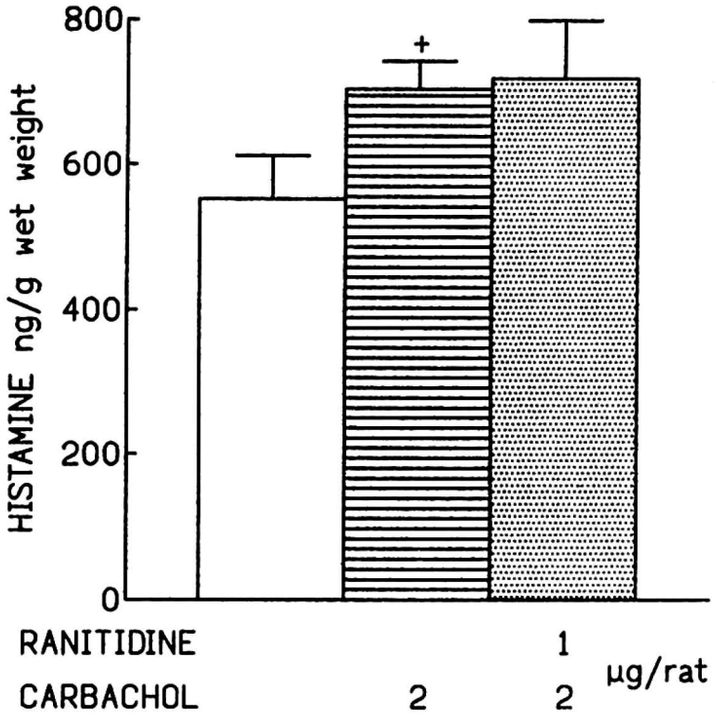

Fig. 6. Hypothalamic histamine content after icv carbachol and ranitidine given 15 min prior to carbachol. $^{+}$ $p < 0.05$ vs. saline controls.

corticosterone secretion is exerted by a mechanism independent of the hypothalamic histamine levels, whereas the effect of mepyramine may be connected with changes in hypothalamic histamine.

DISCUSSION

The present results show that carbachol, a cholinergic muscarinic receptor agonist, given icv dose-dependently stimulates the HPA axis. Total inhibition of the carbachol-induced corticosterone response by icv pretreatment with atropine indicates a fairly selective cholinergic receptor stimulation of the HPA axis by carbachol. Our present results suggest the prevalence of hypothalamic site of a cholinergic muscarinic mechanism in the HPA stimulation by carbachol in conscious rats.

It is also inferred that the level of histamine in the hypothalamus and central histamine receptors may be involved in the carbachol-induced mediation of the HPA axis stimulation. The corticosterone response to carbachol was almost totally abolished by pretreatment of rats with α-FMH, a specific inhibitor of histidine decarboxylase and brain histamine synthesis (13, 14). Since α-FMH did not significantly affect the levels of catecholamines in most brain structures in rats (24), the powerful inhibition of the

carbachol-induced HPA activity by α-FMH may be connected with the reduction of the hypothalamic histamine synthesis. Furthermore, the carbachol-induced increase in the hypothalamic histamine levels found in the present experiment, suggests some role of histamine in mediation of the HPA activity via cholinergic muscarinic receptor stimulation. Mepyramine, a histamine H_1-receptor antagonist, moderately diminished, by 30%, the carbachol-induced corticosterone response. A participation of central histamine H_1-receptors in neostigmine-induced hyperglycemia was found in anesthetized rats (25). Ranitidine, an H_2-receptor antagonist, significantly reduced, by 57%, the rise in corticosterone levels elicited by carbachol. In our former experiment, cimetidine exerted a weaker inhibitory effect than mepyramine (9). However, ranitidine is known to be an H_2-receptor antagonist several times more potent than cimetidine in different reactions mediated by these receptors, including the pituitary hormone secretion (26). Also in the carbachol-induced stimulation of the HPA axis, ranitidine turned out to be a more potent inhibitor than cimetidine.

The above results demonstrate that pretreatment with atropine does not diminish the carbachol-evoked increase in the hypothalamic histamine level, but it almost totally inhibits the carbachol-induced corticosterone response. Similarly, ranitidine does not change carbachol-induced elevation in the hypothalamic histamine content, but it considerably impairs the carbachol-evoked increase in the serum corticosterone concentration. This finding may suggest direct inhibition, via homologous receptor blockade of CRF-secreting neurons, of the carbachol-induced HPA response by these antagonists. The possible stimulation of the CRF release by increased neuronal histamine levels elicited by carbachol may be antagonized by the blocking effect of muscarinic and histamine H_2-receptors on CRF secreting neurons.

The final stimulatory effect of carbachol on the corticosterone secretion may thus involve both the direct stimulation of CRF containing neurons by hypothalamic muscarinic receptors and the increased histamine content in the hypothalamus. Both these actions are selectively blocked by atropine or histamine H_2-receptor antagonists.

The substantial, yet insignificant, diminution of the carbachol-induced corticosterone response by mepyramine was accompanied with the totally inhibited increase in the hypothalamic histamine levels. This finding suggests that only part of the corticosterone level increment elicited by carbachol may be connected with the carbachol-induced increase in the hypothalamic histamine.

In vitro studies did not indicate muscarinic stimulation of the histamine synthesis or its release from the rat brain slices and synaptosomes (18). Muscarinic heteroreceptors, located directly on histaminergic nerve terminals, may control the release and synthesis of histamine in the brain; however

carbachol reduces the K^+-induced histamine release from rat cortical slices *in vitro* (18). In the rat brain, the muscarinic agonist oxotremorine and physostigmine decreased the histamine turnover in the striatum and cerebral cortex, i.e. in the structures where muscarinic receptors are present in a high density. These data suggest that stimulation of central muscarinic receptors presumably inhibits the histaminergic activity in the brain. However, in *in vitro* experiments, the range of drug concentrations used and the brain structures examined by these authors have no close relevance to a possible hypothalamic interference of histamine or its receptors in the carbachol-induced stimulation of the HPA axis in intact, conscious rats.

Part of the increase in the HPA response elicited by carbachol may also be caused by vasopressin, which is coreleased with CRF. Approximately half of the parvocellular CRF neurosecretory perikarya and axon terminals contain vasopressin, and carbachol applied to the paraventricular nucleus induced a severalfold increase in vasopressin secretion (27).

In conclusion, the present results show an involvement of the hypothalamic histamine and central histamine H_1- and H_2-receptors in the carbachol-induced increase of the HPA activity in conscious rats.

Acknowledgements. The authors are very grateful to Dr. J. Kollonitsch, Merck Sharp and Dohme Research Laboratories, for a generous gift of alpha-fluoromethylhistidine.

REFERENCES

1. Calogero AE, Kamilaris TC, Gomez MT et al. The muscarinic cholinergic agonist arecoline stimulates the rat hypothalamic-pituitary-adrenal axis through a centrally-mediated corticotropin-releasing hormone-dependent mechanism. *Endocrinology* 1989; 125: 2445—2453.
2. Hasey G, Hanin I. Plasma corticosterone is increased and correlated with brain acetylcholine in physostigmine — but not in neostigmine — treated rats. *Psychoneuroendocrinology* 1990; 15: 357—369.
3. Suda T, Yajima F, Tomori N et al. Stimulatory effect of acetylcholine on immunoreactive corticotropin-releasing factor release from the rat hypothalamus in vitro. *Life Sci* 1987; 40: 673—677.
4. Janowsky DS, Risch SC. Cholinomimetic and anticholinergic drugs used to investigate an acetylcholine hypothesis of effective disorders and stress. *Drug Develop Res* 1984; 4: 125—142.
5. Tago H, Mc Geer PL, Bruce G, Hersh LB. Distribution of choline acetyltransferase-containing neurons of the hypothalamus. *Brain Res* 1987; 415: 49—62.
6. Barron SE, Hoover DB. Localization of acetylcholinesterase and choline acetyltransferase in the rat pituitary gland. *Histochem J* 1983; 15: 1087—1098.
7. Hauser G, Parks JM. Evidence from phospholipid metabolism changes for muscarinic cholinergic receptors on rat anterior pituitary cells. *J Neurosci Res* 1983; 10: 295—302.
8. Egozi Y, Kloog Y, Fleminger G, Sokolovsky M. Acetylcholine in the rat pituitary: a possible humoral factor. *Brain Res* 1988; 475: 376—379.
9. Bugajski J, Borycz J, Bugajski AJ. Influence of central neuronal histamine on the pituitary-adrenocortical activity stimulated by neurotransmitters. *Agents Actions*, Special Conference Issue 1992; C346—C349.

10. Bugajski J, Gądek-Michalska A, Borycz J, Bugajski AJ. Central histaminergic mechanisms in the corticosterone response to clonidine. *J Physiol Pharmacol* 1993; 44: 303—312.
11. Turoń M, Tytoń J, Bugajski J. The influence of α-fluoromethylhistidine and histamine receptor antagonists on the β-endorphin-induced corticosterone response. *Life Sci* 1991; 48: 1191—1198.
12. Turoń M, Fogel WA, Bugajski J. Alpha-fluoromethylhistidine decreases the leuenkephalinamide-and morfine-induced corticosterone response in rats. *Pharmacology* 1991; 42: 188—196.
13. Kollonitsch J, Patchett AA, Marburg S et al. Selective inhibitors of biosynthesis of aminergic neurotransmitters. Nature 1978; 274: 906—908.
14. Watanabe T, Yamatodani A, Maeyama K, Wada H. Pharmacology of α-fluoromethylhistidine, a specific inhibitor of histidine decarboxylase. *Trends Pharmacol Sci* 1990; 11: 363—367.
15. Bugajski J, Janusz Z. Central histaminergic stimulation of pituitary-adrenocortical response in the rat. *Life Sci* 1983; 33: 1179—1189.
16. Cacabelos R. Histaminergic system: Neuroendocrine function of brain histamine. *Meth Find Exp Clin Pharmacol* 1990; 12: 341—376.
17. Soe-Jensen P, Knigge U, Garbarg M et al. Responses of anterior pituitary hormones and hypothalamic histamine to blockade of histamine synthesis and to selective activation or inactivation of presynaptic histamine H_3 receptors in stressed rats. *Neuroendocrinology* 1993; 57: 532—540.
18. Gulat-Marnay C, Lafitte A, Arrang J-M, Schwartz J-C. Regulation of histamine release and synthesis in the brain by muscarinic receptors. *J Neurochem* 1989; 52: 248—254.
19. Oishi R, Adachi N, Okada K, Muroi N, Saeki K. Regulation of histamine turnover via muscarinic and nicotinic receptors in the brain. *J Neurochem* 1990; 55: 1899—1904.
20. Panula P, Pirvola U, Auvinen S, Ariaksinen MS. Histamine-immunoreactive nerve fibers in the rat brain. *Neuroscience* 1989; 28: 585—610.
21. Glick D, Redlich D, Levine S. Fluorometric determination of corticosterone in 0.02—0.05 milliliters of plasma or sub-miligram samples of adrenal tissue. *Endocrinology* 1964; 74: 653—655.
22. Kremzner LT, Pfeiffer CC. Identification of substances interfering with the fluorometric determination of brain histamine. *Biochem Pharmac* 1966; 15: 197—200.
23. Fogel WA. Enzymatic histamine catabolism in vertebrate ontogenesis. A comparative study. *Comp Biochem Physiol* 1988; 89C: 355—360.
24. Onodera K, Yamatodani A, Watanabe T. Effects of α-fluoromethylhistidine on locomotor activity, brain histamine and catecholamine contents in rats. *Meth Find Exp Clin Pharmacol* 1992; 14: 97—105.
25. Nonogaki K, Iguchi A, Xianzhu L et al. Role of brain histamine H_1-and H_2-receptors in neostigmine-induced hyperglicemia in rats. *Life Sci* 1992; 51: PL 131—134.
26. Netti C, Guidobono F, Olgiati VR, Sibilia V, Pecile A. Comparison of the effects of histamine H_2-receptor antagonists on prolactin secretion in the rat. *Endocrinology* 1983; 113: 412—414.
27. Ota M, Crofton JT, Festavan G, Share L. Central carbachol stimulates vasopressin release into interstitial fluid adjacent to the paraventricular nucleus. *Brain Res* 1992; 592: 249—254.

Received: June 6, 1994
Accepted: July 15, 1994

Author's address: J. Bugajski, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Cracow, Poland