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INVOLVEMENT OF THE CENTRAL NORADRENERGIC SYSTEM IN CHOLINERGIC STIMULATION OF THE PITUITARY-ADRENAL RESPONSE

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Involvement of the central adrenergic system in stimulation of the hypothalamic-pituitary-adrenal (HPA) axis by carbachol, a cholinergic muscarinic agonist, was assessed indirectly through corticosterone secretion. Carbachol (2 μg) given intracerebroventricularly or intraperitoneally evoked a dose-related increase in serum corticosterone levels. On a molar basis, carbachol given icv was considerably more active than when injected ip, indicating its central site of action. The corticosterone response to icv carbachol was significantly reduced by pretreatment of rats 15 min earlier with prazosin, an α_1-adrenergic receptor antagonist. Pretreatment with yohimbine, an α_2-adrenergic antagonists, did not significantly affect the carbachol-induced corticosterone response. Propranolol, a β-adrenergic blocker, given icv or ip significantly impaired the carbachol-elicited corticosterone secretion. The selective noradrenergic neurotoxin DSP-4 (50 mg/kg) given ip 8 days before the experiment, also potently diminished the carbachol-induced rise in serum corticosterone levels. Carbachol markedly increased, while DSP-4 significantly diminished the hypothalamic noradrenaline levels. Likevise, DSP-4 significantly impaired the carbachol-induced rise in hypothalamic noradrenaline levels.

Our present results indicate that the central adrenergic system is involved in the cholinergic muscarinic stimulation of the pituitary-adrenocortical response. Both hypothalamic noradrenaline and adrenergic α_1- and β-receptors are significantly involved in the carbachol-induced HPA response.

Key words: *carbachol, noradrenergic neurotoxin DSP-4, hypothalamic noradrenaline, adrenergic receptor antagonists, corticosterone.*

INTRODUCTION

The central cholinergic system is known to modulate the activity of the hypothalamic-pituitary-adrenal (HPA) axis. Cholinergic agonists stimulate the secretion of corticotropin-releasing hormone (CRH), ACTH and corticosterone (1—3). The increase in plasma corticosterone level was correlated with brain

acetylcholine (4). The anterior pituitary lobe has no nerve supply nor does it contain the acetylcholine-forming enzyme choline acetyltransferase, but it receives stimuli from the circulatory system (5). Intrinsic cholinergic neurons are present in the hypothalamus (6, 7) and muscarinic receptors have been identified in the anterior pituitary (8). Although acetylcholine can stimulate hypothalamic CRH secretion *via* both muscarinic and nicotinic receptor mechanisms (9) this transmitter activates the HPA axis *via* CRH release mainly through muscarinic receptors, since atropine dose-dependently inhibits this stimulation (2, 3). Also muscarinic cholinergic agonists activate the HPA axis in the rat both *in vivo* and *in vitro* mainly by the release of endogenous CRH (1). The existence of an interaction between the cholinergic and the noradrenergic transmission is well established. In the brain, a normal response to acetylcholine depends on the presence of catecholamines. Acetylcholine may exert a different action on dopamine (DA) and noradrenaline (NA) release, depending upon the fact whether muscarinic or nicotinic receptors are activated (10).

Although a cholinergic mechanism itself may be involved in stimulation of CRH and ACTH secretion, changes in the hypothalamic catecholamine neuronal activities may modulate ACTH secretion. It has been shown that neostigmine, a cholinesterase inhibitor, administered into the third cerebral ventricle, acts through central cholinergic muscarinic receptors and stimulates hypothalamic neuronal activities of NA and DA (11). Central activation of the cholinergic system by neostigmine also stimulates the noradrenergic and dopaminergic systems in the hypothalamus (12) and cholinergic drugs have been shown to enhance the synthesis and turnover of brain catecholamines. Also noradrenergic neurons present in the locus coeruleus, which innervates almost the entire brain and spinal cord, can be stimulated *via* cholinergic muscarinic receptors (13).

In the present study we examined the role of central adrenergic receptors and neurons in the carbachol-induced corticosterone secretion in conscious rats.

MATERIALS AND METHODS

Male Wistar rats, weighing 180—200 g, were housed in groups of 6—8 per cage, and fed with food and water ad libitum on a diurnal light cycle at room temperature of 18—21°C, one week prior to the experiment. The experiments were performed in different seasons of the year and no substantial difference in the rat's pituitary-adrenocortical reactivity to different stimuli was observed. Experiments were performed in three groups. In the first group the molar activity of carbachol given icv and ip in stimulation of corticosterone secretionwas examined. In the second group effects of the specific noradrenergic neurotoxin (N-(2-chloroethyl)-N-ethyl-2bromobenzylamine (DSP-4) on the carbachol-stimulated corticosterone secretion and hypothalamic noradrenaline and dopamine levels were examined. For

this purpose rats pretreated with zimelidine (10 mg/kg ip 30 min before DSP-4) to protect 5-HT containing neurons in the central nervous system, were injected with DSP-4 (50 mg/kg) 8 days before icv carbachol. Control rats received ip injection of saline. In the third group rats were treated icv with carbachol, or with α_1, α_2, and β-adrenergic antagonists + carbachol. The drugs were administered into the right lateral cerebral ventricle of rats whose skulls had been prepared earlier under light ether anesthesia for icv injections. Adrenergic antagonists were given 15 min before carbachol. After injection of the drugs the animals were placed back in their cages. One hour after the last injection the rats were decapitated immediately after their removal from cages and their trunk blood was collected. Control animals were injected with the same volume of saline and were decapitated concurrently with the experimental group to obtain resting serum corticosterone levels. The trunk blood was centrifugated and serum aliquots were frozen until a further assay. Serum corticosterone concentration was determined fluorometrically. Corticosterone levels in the serum are expressed as micrograms per 100 ml. One analysis was performed in each rat's serum, but 6 animals were used for each data point. In order to avoid any interference in corticosterone levels by the circadian rhythm all experiments were performed between 9 and 10 a.m., and all decapitations were carried out between 10 and 11 a.m., i.e. when the serum corticosterone concentration is low in the normal diurnal rhythm.

For a HPLC assay, the brains were quickly removed and the hypothalami were dissected on a cooled plate and immediately frozen on dry ice. The frozen tissue samples were placed into approx. 10 vol. of ice-cold 0.1 M. $HClO_4$ containing 5 mM of ascorbic acid and 25 μg/l of 3,4 dihydrobenzylamine (internal standard), weighed and homogenized with an Ultra-Turrax homogenizer (10 s at 20000 rpm). The homogenates were centrifuged at 14000 × g and the supernatants were subsequently filtered out through 0.22 μm. RC-58 membranes (BAS MF-1 centrifugal microfilters). The filtrates were injected into the HPLC system. A BAS-400 liquid chromatograph was used (BAS, USA), equipped with an LC4B/17AT electrochemical detector and 3 μm. C_{18} Phase 2 analytical column (100 mm × 3 mm) which was coupled with 7 μm. C_{18} guard column (15 mm × 3 mm). The mobile phase (36 mM citrate-28 mM phosphate buffer pH 3.5, containing 0.77 mM of EDTA and 5% methanol) was pumped at 0.9 ml/min through the column thermostated at 32°C. The separated sample components of noradrenaline were detected at an oxidation potential of 0.8 V. All the reagents were of analytical grade (Merck, Germany and Sigma, USA).

The following drugs were used: N-(2-chloroethyl-)N-ethyl-2-bromobenzylamine (DSP-4) and zimelidine (Astra, Sodertalje), carbamylcholine hydrochloride (carbachol), DL-propranolol hydrochloride and yohimbine (Sigma) and prazosin hydrochloride (Pfizer). All drug solutions for an icv administration were prepared in 10 μl of sterile saline and those for ip injection in a volume of 1 ml/kg.

The data are presented as arithmetical means and standard errors of the means. The significance of differences between the groups was assessed by an analysis of variance followed, if necessary, by a specific comparison with the Duncan test.

RESULTS

Effect of carbachol on corticosterone secretion

Carbachol given icv or ip evoked a dose-dependent rise in serum conticosterone levels, measured 1 h after injection. However, carbachol administered icv exhibited a considerably more potent molar activity than when it was injected systematically (*Fig. 1*).

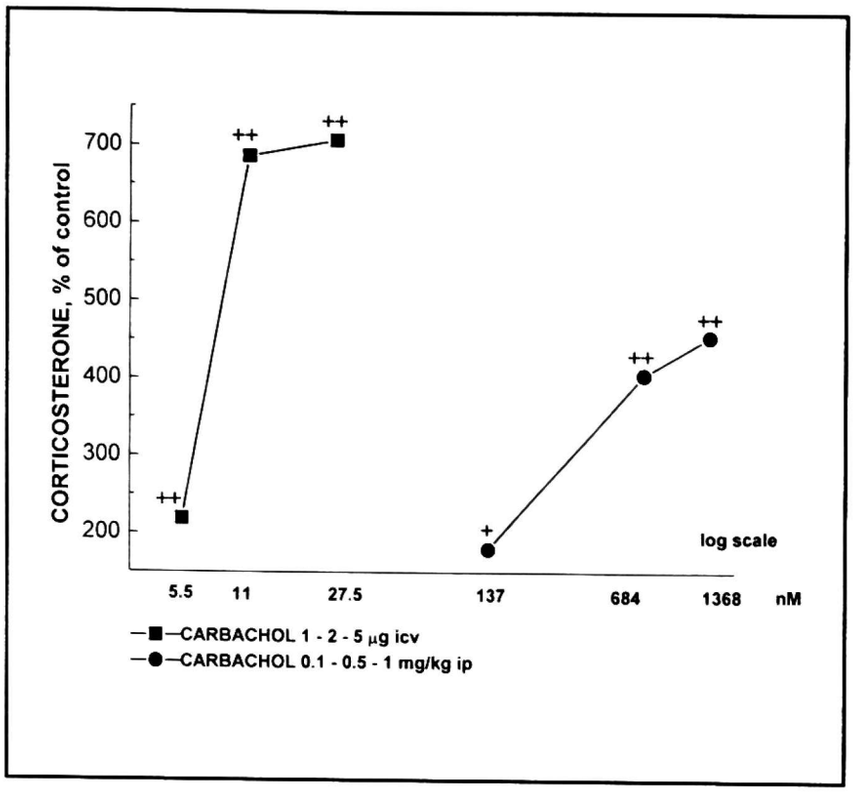

Fig. 1. Corticosterone secretion 1 h after ip and icv administration of carbachol. Log dose-response values of 6—8 rats.

Effect of adrenergic antagonists on carbachol-induced corticosterone secretion

Adrenergic receptor antagonists given icv 15 min prior to carbachol markedly diminished the carbachol-induced corticosterone secretion. Prazosin (0.01 and 0.1 µg), an α_1-adrenergic receptor antagonist, considerably decreased the carbachol-induced corticosterone response, by 33 and 56%, respectively (*Fig. 2*). Pretreatment with yohimbine (0.1 and 1 µg), an α_2-adrenergic antagonist, induced a moderate and statistically not significant diminution of the carbachol-elicited corticosterone secretion, by 21 and 18%, respectively (*Fig. 3*). Propranolol, a β-adrenergic receptor antagonist, given both icv and ip (10 µg/rat and 0.1 mg/kg) impaired potently and to a similar extent the carbachol-induced corticosterone response, by 58 and 54%, respectively (*Fig. 4*).

Effect of the adrenergic neurotoxin DSP-4 on carbachol-induced corticosterone secretion and hypothalamic catecholamine levels

The specific adrenergic neurotoxin DSP-4 (50 mg/kg ip) given 8 days before experiment considerably diminished, by 61%, the carbachol-induced corticosterone secretion (*Fig. 5*). Carbachol (2 µg icv) induced a moderate but consistent increase in hypothalamic catecholamine levels. The levels of noradrenaline and dopamine in that structure rose by 10 and 8%, respectively, 1 h after carbachol injection. DSP-4 significantly decreased hypothalamic NA level in both control and carbachol-treated rats (*Table 1*).

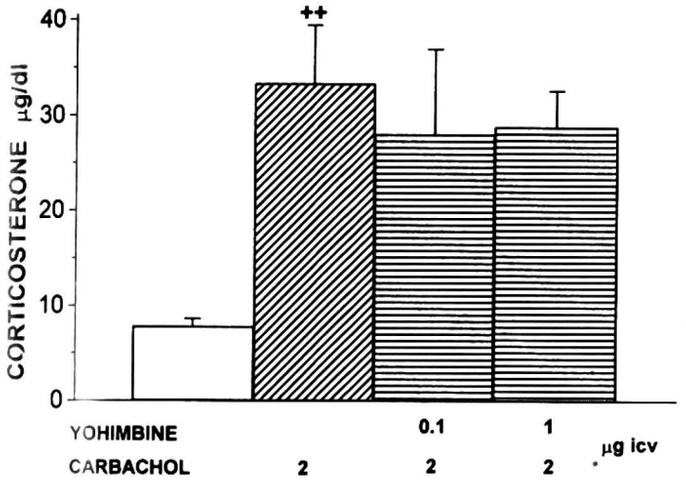

Fig. 2. Effect of prazosin on the carbachol-induced corticosterone secretion. Prazosin was administered icv 15 min prior to carbachol and 1 h later the rats were decapitated. In *Fig. 2—5* values represent the mean of 6 rats. $^{++}p < 0.01$ vs. saline control, $^{**}p < 0.01$ vs. carbachol-treated group.

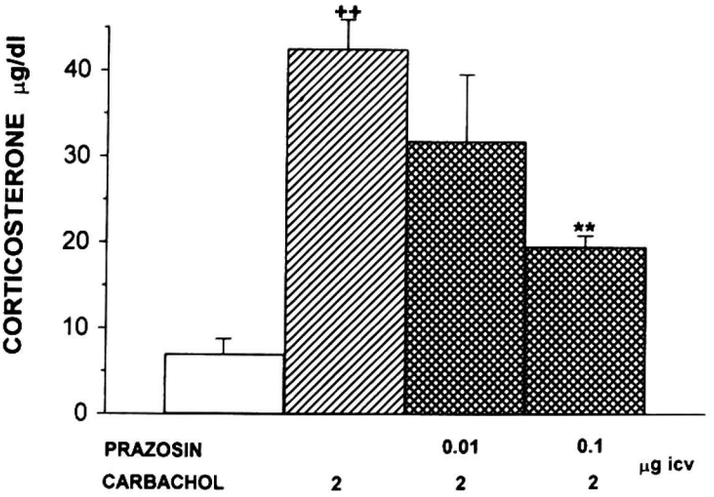

Fig. 3. Effect of yohimbine on the carbachol-induced corticosterone secretion. Yohimbine was administered icv 15 min prior to carbachol and 1 h later the rats were decapitated. $^{++}p < 0.01$ vs. saline control.

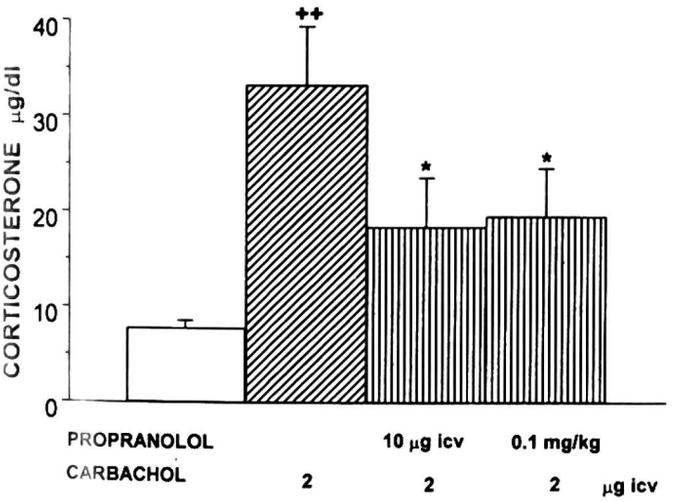

Fig. 4. Effect of propranolol on the carbachol-induced corticosterone secretion. Propranolol was injected icv or ip 15 min before carbachol. $^{++}p < 0.01$ vs. saline control, $^{*}p < 0.05$ vs. carbachol-treated group.

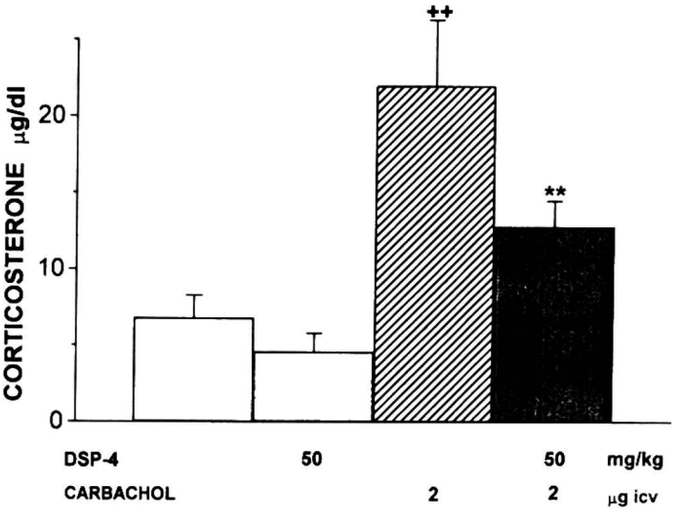

Fig. 5. The influence of DSP-4 (50 mg/kg ip 8 days before carbachol) on the carbachol-induced corticosterone secretion. $^{++}p < 0.01$ vs. saline control, $^{**}p < 0.01$ vs. carbachol-treated group.

Table 1. Effect of carbachol and DSP-4 on hypothalamic noradrenaline and dopamine levels. DSP-4 (50 mg/kg ip) was injected 8 days before experiment. One hour after carbachol administration the rats were decapitated and their hypothalami isolated for HPLC assay. $^{+}p < 0.01$ vs. saline control, $^{*}p < 0.01$ vs. carbachol-treated group.

TREATMENT	NORADRENALINE ng/g wet weight	DOPAMINE ng/g wet weight
NaCl	2578 ± 152	490 ± 30
DSP-4	2047 ± 106 $^{+}$	452 ± 113
CRB 2 µg icv	2908 ± 242	529 ± 63
DSP-4 + CRB	2112 ± 242 *	603 ± 97

DISCUSSION

The present results show that, on a molar basis, carbachol administered icv is considerably more potent than when given systemically in stimulating corticosterone secretion. This finding clearly corroborates the hypothalamic site of action of carbachol in stimulation of the HPA axis. It is unlikely that carbachol in a dose given icv (2 μg) in the present experiment can penetrate, *via* portal circulation, the anterior hypophysis to activate directly muscarinic receptors (5) and to stimulate ACTH secretion. Carbachol administered icv stimulates *via* muscarinic receptors, the CRH secretion from the hypothalamic paraventricular nucleus (1). Carbachol can stimulate both muscarinic and nicotinic acetylcholine receptors to activate the HPA axis. However, the muscarinic component is mainly responsible for the observed stimulation since atropine, a muscarinic antagonist, abolished (3) and mecamylamine, a nicotinic antagonist, did not affect (data not shown) the carbachol-induced corticosterone secretion.

Although central cholinergic stimulation itself may activate CRH and ACTH secretion, changes that accompany hypothalamic catecholaminergic activities may modulate this secretion. Our results clearly show an involvement of the central adrenergic system in the carbachol-elicited corticosterone response. Intraventricular pretreatment with prazosin, an α_1-adrenergic receptor antagonist, diminished, by 56%, the carbachol-induced corticosterone response. This observation may suggest that activation of cholinergic muscarinic receptors by carbachol also stimulates the hypothalamic noradrenergic system which is considerably impaired by the α_1-adrenergic blocker. A similar significant increase in ACTH secretion and hypothalamic noradrenaline activity was observed by Gotoh *et al.* (11) 1 h after icv neostigmine, a cholinesterase inhibitor. Yohimbine (0.1 and 1 μg icv) given prior to carbachol did not markedly affect the carbachol-induced corticosterone response. Only small diminution of that response was observed. This finding suggests that cholinergic muscarinic stimulation does not interfere with presynaptic adrenergic receptors which are known to be involved in regulation of the HPA activity (14, 15). Our results also show that β-adrenergic receptors are significantly involved in mediation of the muscarinic receptor-stimulated HPA response. Propranolol, a β-adrenergic antagonist, given icv or systemically reduces the carbachol-elicited corticosterone secretion, by 58 and 54%, respectively. This observation suggests the involvement of the noradrenergic system in carbachol-induced corticosterone secretion. Our results confirm the thesis that the response to acetylcholine depends on the presence of catecholamines. Noradrenaline liberated by muscarinic stimulation can activate hypothalamic α_1- and β-adrenergic mechanisms to facilitate CRH secretion from the paraventricular nucleus. We

showed previously that both α_1- and β-adrenergic receptors equally participated in stimulation of the HPA axis by noradrenaline (16).

Also cardiovascular responses to brain cholinergic stimulation were found to be mediated by central catecholaminergic activation (17). Carbachol microinjected into the posterior hypothalamic nucleus of conscious rats increased the mean arterial pressure which was partly attenuated by prazosin, an α_1-adrenoceptor blocker or yohimbine, an α_2-adrenoceptor blocker (18).

Our data show that in rats pretreated with DSP-4 (50 mg/kg ip) 8 days before the experiment the carbachol-induced corticosterone response is significantly diminished, by 61%, in comparison with such a response in saline-treated controls. However, this diminution is weaker (by 21%) than the decrease in the hypothalamic NA level. DSP-4 used in higher doses (100 mg/kg ip) was able to reduce the hypothalamic NA level by 83% three days after injection and that reduction was drastically diminished after 15 days (19). It is well known that the locus coeruleus noradrenergic system communicates with the CRH system *via* the ascending noradrenergic bundle and is involved in regulation of the HPA axis. DSP-4 is capable of selectively depleting noradrenergic axons and subsequently inducing lesions of locus coeruleus noradrenergic neurons (20) causing reduction of hypothalamic noradrenaline which may mediate part of the carbachol-induced HPA stimulation. DSP-4 also caused significant diminution of hypothalamic noradrenaline levels in both basal and stress conditions in rats whose HPA system was stimulated by CRH and vasopressin (21).

Our present results indicate that the central adrenergic system is involved in cholinergic muscarinic stimulation of the pituitary-adrenocortical response. Both hypothalamic noradrenaline and adrenergic α_1- and β-receptors are significantly involved in the carbachol-induced HPA response.

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