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EFFECT OF INDOMETHACIN ON NICOTINE-INDUCED ACTH AND CORTICOSTERONE RESPONSE

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The effect of nicotine on ACTH and corticosterone secretion and possible mediation of prostaglandins in this secretion was investigated in conscious rats. Nicotine (5 and 10 mg/kg ip) considerably increased the plasma ACTH and corticosterone levels, measured 1h after injection. Mecamylamine (10 and 50 μg icv), a nicotinic receptor antagonist, given 15 min prior to nicotine dose-dependently diminished the ACTH and corticosterone responses, by 59 and 30% respectively. Pretreatment with hexamethonium (2 mg/kg ip), a peripheral blocker of nicotinic receptors, diminished to a similar extent the nicotine-induced ACTH and corticosterone responses. On the other hand atropine, a muscarinic receptor antagonist, did not markedly alter those responses. Systemic or intracerebroventricular pretreatment with indomethacin (2 mg/kg ip or 0.1 and 1 μg icv), a cyclooxygenase and endogenous prostaglandin synthesis blocker considerably reduced, by 58%, the nicotine-induced ACTH response, but did not alter the corticosterone response. These results show that nicotine given systemically stimulates ACTH and corticosterone secretion by selective activation of central and peripheral acetylcholine nicotinic receptors. Endogenous prostaglandins are significantly involved in the nicotine-induced central stimulation of ACTH secretion. Prostaglandins do not directly affect the nicotine-induced corticosterone secretion from the adrenal cortex.

Key words: *nicotine, ACTH, corticosterone, indomethacin, prostaglandins.*

INTRODUCTION

High-affinity nicotinic acetylcholine binding sites are widely distributed in specific regions of the central nervous system, and most of them have also been shown to possess functional nicotinic receptors (1—3). The nicotinic receptors have been implicated in various physiological and pathological conditions. These receptors are also present in brain structures and pathways which regulate the release of several pituitary hormones, including ACTH and vasopressin (4—7). The parovocellular region of the hypothalamic

paraventricular nucleus (PVN) is the major site where coricotropin-releasing hormone (CRH) is synthesized and then transported to axon terminals in the median eminence (8). CRH-secreting neurons in the hypothalamus have been assumed to contain nicotinic cholinergic receptors. Immunoreactive nicotinic acetylcholine receptors are also present in axon terminals of the median eminence in the rat. This indicates that nicotine may act on nicotinic acetylcholine receptors to release CRH (9) and ACTH from anterior pituitary corticotrops and subsequently corticosterone from the adrenal gland. Nicotine stimulates CRH secretion from hypothalamic slices. This stimulation is prevented by atropine and hexamethonium, which suggests that both nicotinic acetylcholine receptors and muscarinic acetylcholine receptors are involved in CRH secretion. On the other hand mecamylamine, a selective nicotinic receptor antagonist, totally inhibits the ACTH and β-endorphin response to icv choline, whereas atropine, a muscarinic receptor antagonist, fails to alter these responses. Thus choline can elevate plasma ACTH and β-endorphin levels through activation of central nicotinic receptors (10). In the isolated perfused mouse brain nicotine stimulates the secretion of β-endorphin and ACTH. However, nicotine has no direct effect on the secretion from isolated superfused pituitaries (11) which suggests a hypothalamic site of action.

In the CNS prostaglandins maintain neuroregulatory functions (12). Prostaglandins are known to be involved in regulation of the HPA activity stimulated by neurotransmitters and neuropeptides (13). We have found that prostaglandins significantly mediate the HPA activity induced by central activation of α_1- and α_2-adrenergic receptors, but not by β-adrenergic receptors (14). Possible involvement of prostaglandins in the HPA response stimulated by nicotinic receptors is not known as yet. The purpose of the present study was to evaluate the HPA response to nicotinic receptor stimulation. The other objective was to find out whether endogenous prostaglandins were involved in that stimulation.

MATERIALS

All experiments were performed on adult male Wistar rats weighing 200—230 g. One week before the experiment the rats were housed in an animal room 6 per cage at a temperature of 21°C, under normal day-night cycle lighting conditiona. They had free access to food and tap water. Drugs were injected ip in a volume of 0.2 ml/kg or were administered into the right lateral cerebral ventricle in a volume of 10 μl to the animals whose skulls were prepared one day earlier for free-hand injections under light ether anesthesia.

Nicotine was injected intraperitoneally. Cholinergic antagonists and the cyclooxygenase inhibitor indomethacin were injected 15 min before nicotine. Mecamylamine was administered icv, atropine and hexamethonium ip an indomethacin by either route. Control animals received simultaneously 0.2 ml or 10 μl of saline and were left undisturbed in their cages until decapitation, concurrently with the animals injected with the drugs. One hour after the last injection the rats

were killed by rapid decapitation, and their trunk blood was collected into iced EDTA tubes, immersed in an ice cold bath at 2—8°C, and the plasma was separated by centrifugation in a refrigerated centrifuge. The samples were stored frozenat −20°C until the assay. Plasma ACTH concentrations were measured in duplicates using the double antibody ^{125}I radioimmunoassay obtained from Diagnostic Product Corporation. For corticosterone determinations, aliquots were frozen at −70°C until the assay. The concentration of corticosterone was measured fluorometrically. To avoid ACTH and corticosterone fluctuations due to the circadian rhythm all experiments were performed between 9—11 h, and all decapitations took place between 11—12 h.

Drugs used: L-nicotine, mecamylamine hydrochloride, atropine sulphate, hexamethonium and indomethacin were obtained from Sigma. All drugs were dissolved immediately before use. Indomethacin was dissolved in a 4% Na_2CO_3 solution, and the remaining drugs in 0.9% NaCl solution.

Statistical analysis. All results are expressed as means ±SEM. Statistical probabilities were calculated by an analysis of variance, followed by individual comparisons with the Duncan test. The probability value < 0.05 indicates a statistically significant difference between group means.

RESULTS

Effect of nicotine on plasma ACTH and corticosterone levels

Nicotine injected ip in doses of 5 and 10 mg/kg elevated considerably and to the same extent the plasma ACTH level at 1h after injection. The increase in the plasma corticosterone level by 268 and 330% after 5 and 10 mg/kg of nicotine, respectively, was related to the administered dose (*Fig. 1*).

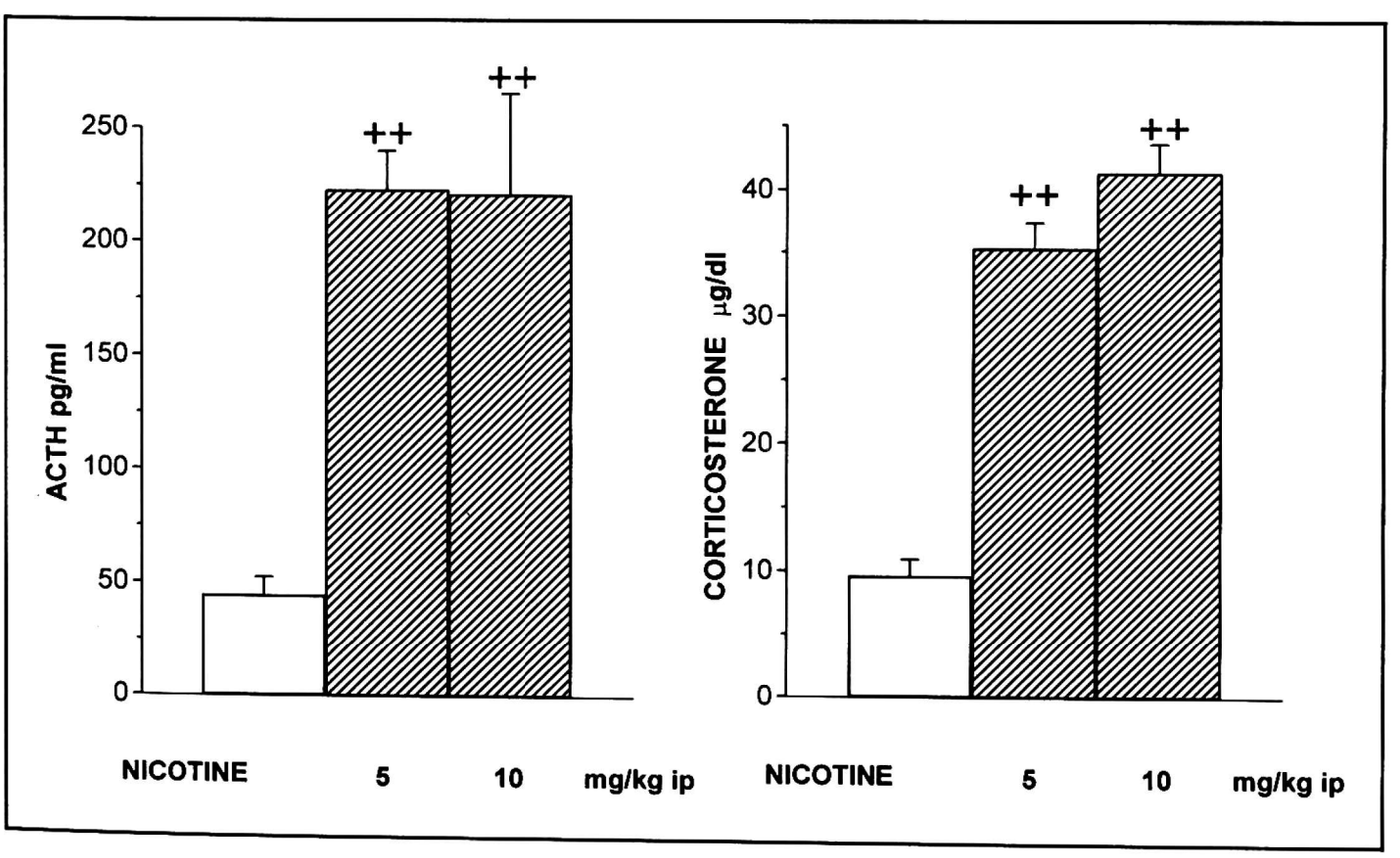

Fig. 1. Effect of nicotine on serum ACTH and corticosterone levels. Nicotine was injected 1 h before decapitation. *In Fig.* 1—5 values represent the mean ±SEM of 6 rats. $^{++}p < 0.01$ vs. saline controls, $*p < 0.05$ and $**p < 0.01$ vs. nicotine-treated group.

Effect of nicotine receptor antagonists on nicotine-induced hormone secretion

In order to determine a central cmponent of the HPA response to systemically injected nicotine, the specific nicotine receptor antagonist mecamylamine was administered icv 15 min prior to nicotine. That antagonist (10 and 50 μg) significantly and dose-dependently reduced the nicotine-elicited ACTH response by 25 and 59%, respectively. Similary, though to a lesser extent, mecamylamine (10 and 50 μg) also decreased the nicotine-induced corticosterone response by 16 and 30%, respectively (*Fig. 2*).

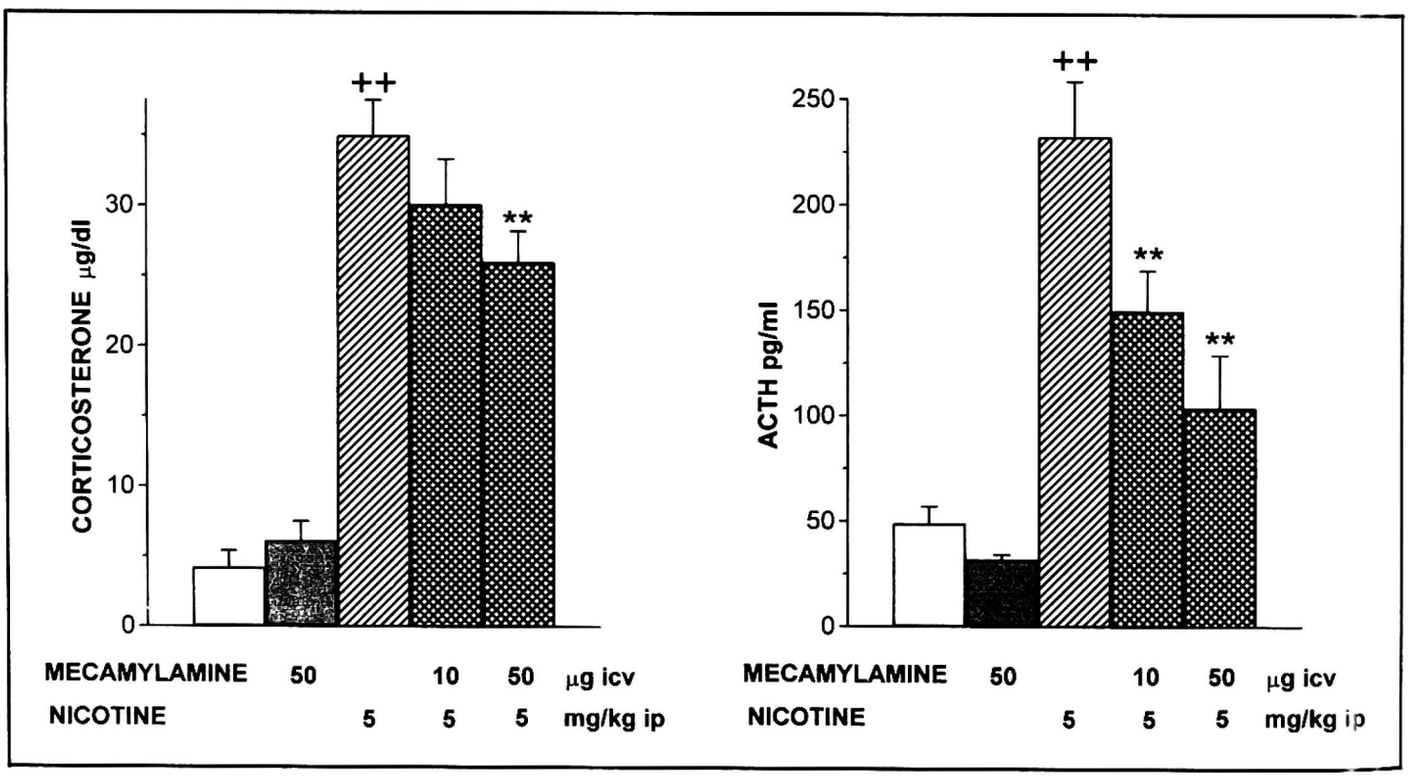

Fig. 2. Effect of mecamylamine on ACTH and corticosterone secretion induced by nicotine. Mecamylamine was injected icv 15 min before nicotine. See legend to *Fig. 1.*

Hexamethonium, a peripherally acting nicotinic antagonist, in the most effective dose of 2 mg/kg ip significantly reduced the nicotine-induced ACTH and corticosterone response, by 54 and 40%, respectively (*Fig. 3*). In contrast, atropine (0.1 mg/kg) a muscarinic receptor antagonist, in the dose that totally abolished the carbachol-induced hormonal responses, did not alter the ACTH response and moderately (by 23%) diminished the corticosterone response induced by nicotine (*Fig. 3*).

Effect of indomethacin on nicotine-induced responses

Indomethacin (2 mg/kg), a cyclooxygenase and prostaglandin synthesis inhibitor, given ip in a dose which in our former experiments most effectively blocked the HPA activation by different stimuli also considerably reduced, by

58%, the nicotine-elicited ACTH response, but did not alter the concomitant corticosterone secretion (*Fig. 4*). Likewise, indomethacin (0.1 and 1 μg given icv) significantly diminished the nicotine-induced ACTH secretion, up to 56% after the lower dose. Indomethacin given icv did not markedly affect the nicotine-elicited increase in corticosterone secretion (*Fig. 5*).

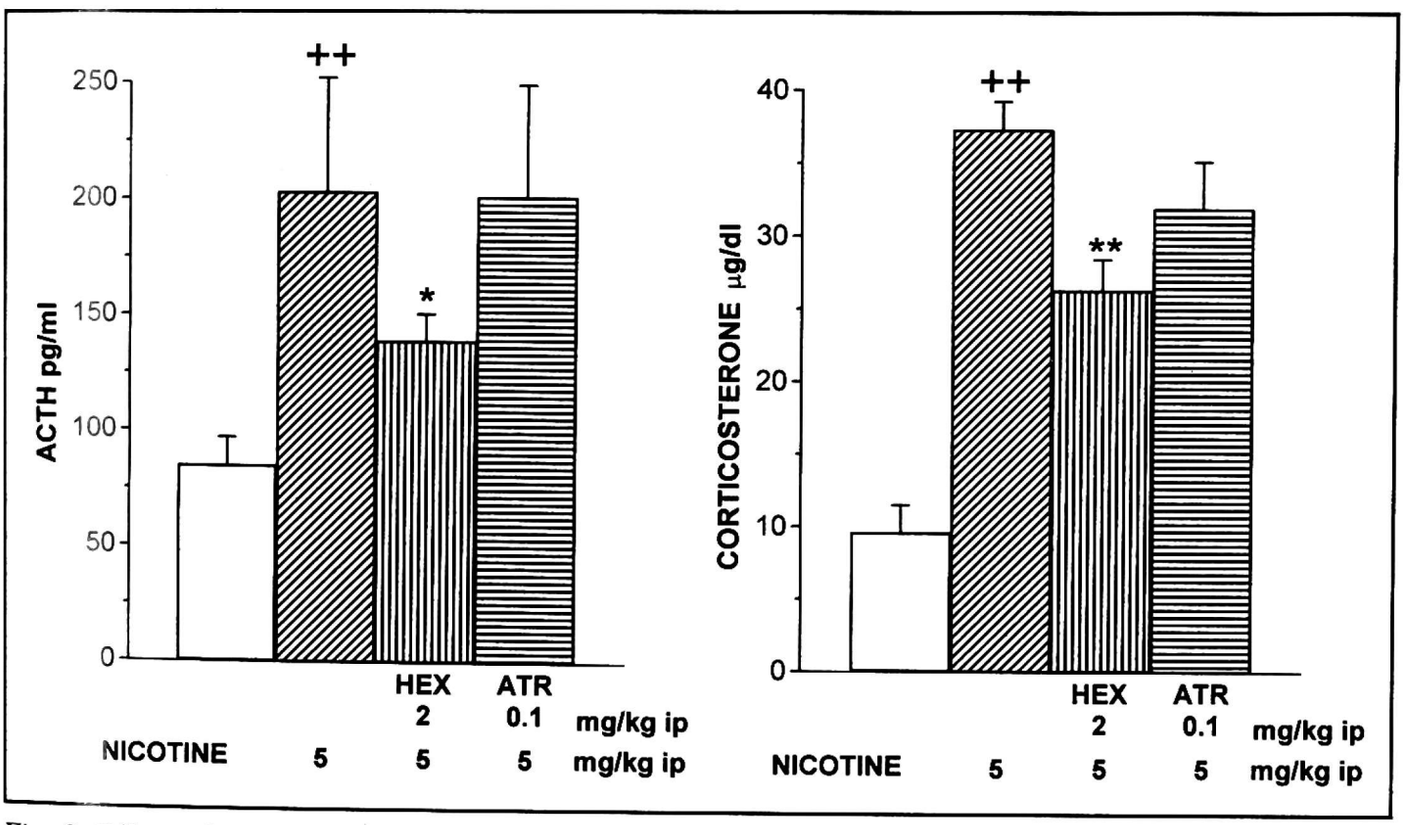

Fig. 3. Effect of hexamethonium and atropine on ACTH and corticosterone secretion induced by nicotine. Hexamethonium and atropine were injected 15 min before nicotine. See legend to *Fig. 1.*

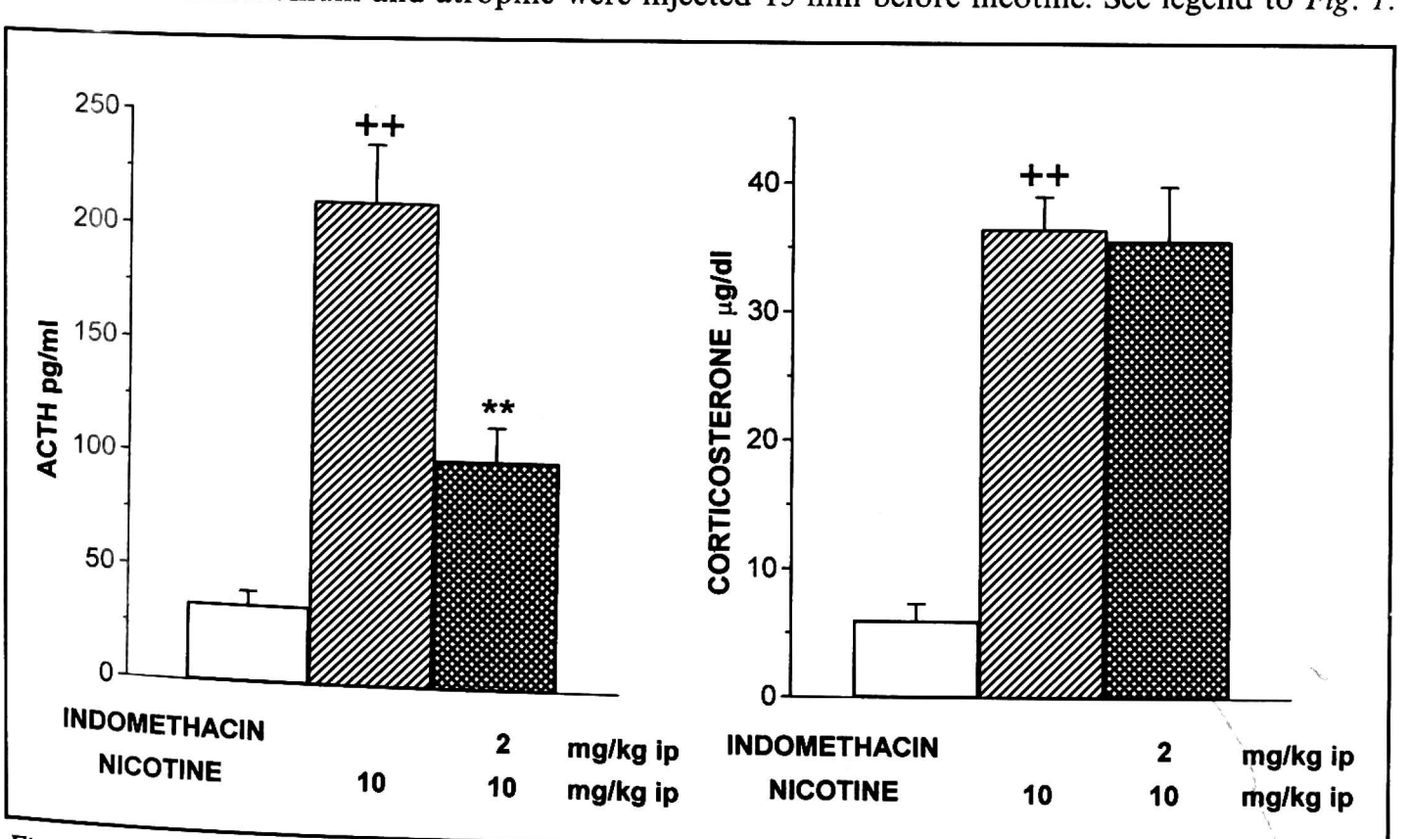

Fig. 4. Effect of indomethacin on nicotine-induced ACTH and corticosterone secretion. Indomethacin was injected ip 15 min before nicotine. See legend to *Fig. 1.*

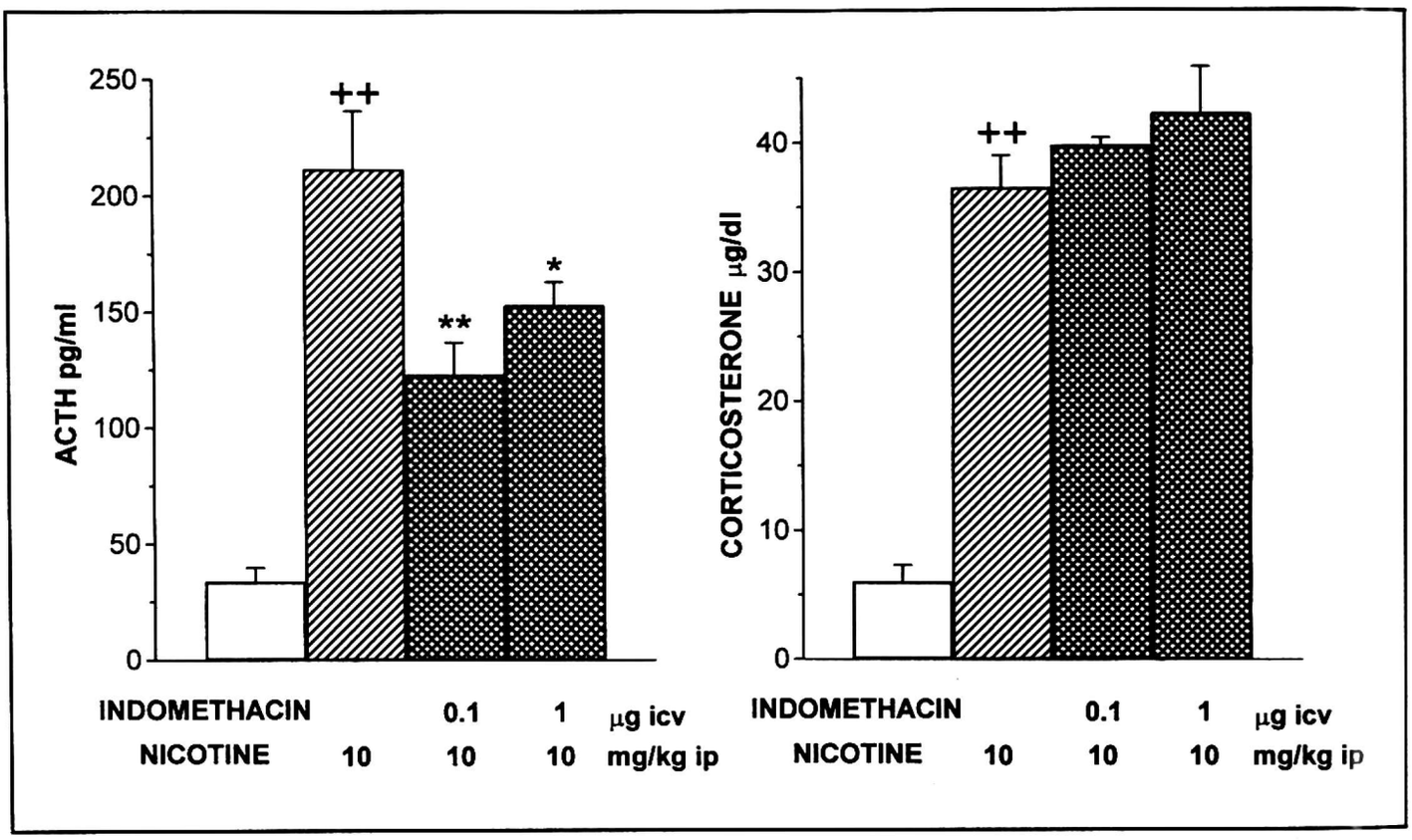

Fig. 5. Effect of indomethacin on nicotine-induced ACTH and corticosterone secretion. Indomethacin was injected icv 15 min before nicotine. See legend to *Fig. 1.*

DISCUSSION

In the present study nicotine given systemically considerably increased the plasma ACTH and corticosterone levels. The maximum ACTH response was elicited by a dose of 5 mg/kg, whereas plasma corticosterone was higher after a dose of 10 mg/kg. Nicotine injected ip stimulated predominantly central nicotine receptors, since icv pretreatment with mecamylamine, a selective nicotinic receptor antagonist, reduced by 59% the nicotine-induced ACTH response. This reduction may have resulted from mecamylamine-evoked inhibition of the nicotine-induced release of CRH from hypothalamic PVN neurons. Mecamylamine also diminished, by 30%, the nicotine-induced corticosterone secretion i.e. to a lesser extent than the ACTH secretion. The above results suggest a predominantly central site of the stimulatory action of nicotine on the HPA axis, since nicotine did not directly interact with the adrenal cortex. Although weaker, inhibition of the corticosterone secretion by mecamylamine followed significant reduction in the ACTH secretion. Nicotine microinjected into catecholaminergic regions of rat brainstem stimulated ACTH secretion (15) and injected ip it induced alterations in the catecholamine concentration in brain structures (16). Those changes were antagonized by mecamylamine, but not by hexamethonium. This suggests that in the present experiment central catecholamines, which are known to stimulate the HPA axis (17), participated in the nicotine-induced increase of ACTH secretion. Hexamethonium (2 mg/kg ip) also diminished, by 54%, the nicotine-induced

ACTH response. Although hexamethonium, a quarternal ammonium compound, does not easily penetrate the CNS through the blood-brain barrier, it may block nicotinic receptors in the median eminence and partly inhibit the CRH release and ACTH secretion. That peripheral nicotinic receptor antagonist decreased more effectively (by 40%) the nicotine-induced corticosterone secretion than did mecamylamine. It is not clear whether this inhibition may reflect a direct action of hexamethonium on the adrenal cortex. In contrast, atropine, a muscarinic receptor antagonist, did not affect the nicotine-induced ACTH release and only moderately diminished corticosterone secretion. These results indicate that muscarinic acetylcholine receptors are not involved in stimulation of the HPA axis by nicotine at either the central or the adrenal gland level as was shown during CRH secretion from hypothalamic slices.

The present results clearly show that endogenous prostaglandins significantly participate in the nicotine-elicited ACTH secretion. Systemic pretreatment with indomethacin (2 mg/kg), a nonselective blocker of cyclooxygenase, in a dose that effectivelly blocks the HPA response to adrenergic (14) and vasopressin stimulation (18),considerably reduces, by 58%, the nicotine-induced ACTH secretion. Indomethacin does not easily penetrate the blood-brain barrier from peripheral circulation, but it may act directly on the median eminence and/or the anterior hypophysis to inhibit CRH and ACTH release. Indomethacin given ip may also penetrate, *via* fenestrated capillaries of the circumventricular organs, the hypothalamic PVN region and inhibit CRH release, Since identical inhibition, by 56%, of ACTH secretion was evoked by indomethacin given icv, a hypothalamic site of the interaction with nicotinic stimulation is strongly suggested. Therefore our present results show that prostaglandins mediate the nicotine-induced ACTH secretion which is considerably impaired by the indomethacin-induced inhibition of prostaglandin synthesis. It is unknown at present whether and to what extent the central adrenergic system is involved in mediation by prostaglandins of the nicotine-induced ACTH secretion. It has been shown that central α_1-and α_2-adrenergic receptors are involved in the ACTH response to nicotine (19). Moreover, our earlier results indicated involvement of prostaglandins in the α_1-and α_2-receptor stimulated HPA activity by phenylnephrine and clonidine, respectively (18, 20). It is therefore very likely that the inhibitory effect of indomethacin on the nicotine-induced ACTH secretion, observed in the present experiment, may be partly connected with reduction of the α_1- and α_2-adrenergic receptor stimulation by nicotine.

Indomethacin given ip or icv did not substantially alter the nicotine-elicited corticosterone response. This finding indicates that prostaglandins are not involved in the nicotine-induced corticosterone secretion at the adrenal gland level. We observed a similar lack of effect of indomethacin on the

corticosterone response to ip carbachol which stimulates the HPA activity predominantly *via* muscarinic receptors (21). Together, our results indicate that endogenous prostaglandis significantly mediate the nicotine-induced ACTH secretion, but are not involved in the muscarinic- or nicotinic receptor-mediated corticosterone secretion from the adrenal cortex in conscious rats.

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