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CALCIUM CHANNEL BLOCKERS IMPAIR THE PITUITARY-ADRENOCORTICAL RESPONSES TO CENTRAL ADRENERGIC RECEPTORS STIMULATION

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The role of calcium channels in the hypothalamic-pituitary-adrenal (HPA) activity, stimulated by adrenergic receptor agonists was investigated indirectly through corticosterone secretion in conscious rats. The drugs were given intracerebroventricularly (icv) or intraperitoneally, calcium channel blockers 15 min before adrenergic receptor agonists. Verapamil and nifedipine considerably diminished the increase in serum corticosterone level induced by icv phenylephrine, an α_1-adrenergic receptor agonist. Verapamil almost completely suppressed the corticosterone response to icv clonidine, an α_2-adrenergic receptor agonist, and abolished the corticosterone response to the centrally administered isoproterenol, a β — adrenergic agonist.
The results demonstrate a significant role of calcium channels and calcium ions in the HPA activity, stimulated centrally by adrenergic receptor agonists. They also indicate that verapamil and nifedipine interfere predominantly with calcium channels on the hypothalamic CRH secreting neurons and anterior pituitary corticotrops when inhibiting pituitary-adrenocortical activity stimulated by adrenergic receptor agonists.

Key words: *Calcium channels antagonists, verapamil, nifedipine, adrenergic agonists, pituitary-adrenocortical activity, corticosterone.*

INTRODUCTION

Calcium ions, which enter cells through either voltage-dependent calcium channels or receptor-activated channels operated via the adenylate cyclase-cAMP system, are necessary for coupling excitation to secretion (1).

Particularly important for the function of secreting cells are receptor-operated channels in the plasma membrane which open in response to a hormone-receptor interaction without a change in the membrane potential (2).

It is well known that calcium ions play an important role in the release of hormones from the anterior hypophysis (3-5) and adrenal glands (6). However, the calcium channel blocker verapamil did not inhibit the basal release of ACTH *in vitro*, but caused powerful and prolonged inhibition of stimulated with ACTH corticosterone production by adrenal tissue. There is convincing evidence for the presence and participation of voltage-sensitive calcium channels in the secretion of all five populations of anterior pituitary cells including corticotrops (7). Nifedipine is capable of inhibiting the stimulated ACTH and cortisol release in humans probably due to blockade of plasma membrane calcium channels of pituitary corticotrops (8). Although the action of CRH, the most important stimulus of ACTH secretion, is mediated by cAMP, the extent to which the ACTH release is modulated by extracellular calcium and calcium channels is still unknown. Giguere et al. (9) showed that the organic calcium blocker verapamil did not inhibit the spontaneous CRH-mediated ACTH secretion whereas other authors demonstrated that Ca^{2+} channels were involved in both the basal and CRH-stimulated ACTH release from anterior pituitary corticotrops (10). Also Aguilera et al. (11) reported a decrease in the maximum amount of ACTH released after exposure to CRH and dihydropyridine nifedipine. The CRH-mediated ACTH release may be augumented by activation of calcium-dependent systems. These data suggest that full activation of the ACTH release may require extracellular calcium ions. The adrenal cortisol response to ACTH appears to involve both calcium and cAMP as intracellular mediators. In humans, nifedipine had no effect on the basal or peak cortisol levels, but reduced the incremental hormone response to ACTH (12).

The site and mechanism of action of calcium ions and calcium channels in the hypotalamic-pituitary-adrenal activity, stimulated by adrenergic receptor agonists, remain unclear.

Calcium channel blockers, though used predominantly as antiarrhytmic, antihypertensive and antianginal agents and also to treat affective disorders (13), probably cross the blood-brain barrier and are likely to affect the activity of the hypothalamic-pituitary-adrenal axis, which is significantly controlled by the adrenergic system (14).

The present study was designed to investigate the significance of the calcium channel blockers verapamil and nifedipine in the hypothalamic-pituitary-adrenocortical activity stimulated by adrenergic receptor agonists.

MATERIALS AND METHODS

Adult male Wistar rats weighing 190-220 g, with free access to food and water, were used in all experiments. The animals were kept in group cages under standard laboratory conditions on a diurnal light cycle, at least one week prior to experimentation. The rats were arbitrarily assigned to one of the experimental groups. The indicated doses of drugs were injected either into the right

lateral cerebral ventricle in a volume of 10 µl of saline or intraperitoneally in a volume of 1 ml/kg. Control rats received 10 µl or 0.2 ml of saline, respectively. Calcium channel antagonists were administered 15 min before adrenergic receptor agonists. One hour after the last injection, the rats were decapitated and their trunk blood was collected. The control rats were decapitated concurrently with the experimental group. After centrifugation, serum aliquots were frozen until the assay. The serum corticosterone concentration was determined fluorometrically (15) and expressed as µg per 100 ml. One analysis was performed in each rat's serum, but 7 animals were used for each point. All the experiments were performed between 9 and 12 a.m., and all decapitations between 11 and 12 a.m. to avoid corticosterone level fluctuations due to the diurnal rhythm.

Drugs

The following drugs were used: L-phenylephrine hydrochloride, DL-isoproterenol hydrochloride, (+−) verapamil hydrochloride (Sigma) and clonidine (Boehringer).

The drugs were dissolved in sterile saline immediately before use and nifedipine was suspended in 1% Tween 80.

The results were calculated as a group mean ± standard error of the mean. The statistical evaluation was performed by an analysis of variance, followed by individual comparisons with Duncan's test.

RESULTS

1. Effect of calcium channel blockers on resting serum corticosterone levels

Verapamil and nifedipine, given either centrally or systemically in the doses used in these experiments as calcium channel blockers, did not significantly change the resting serum corticosterone levels (*Table 1*).

Table 1. Effect of verapamil and nifedipine on resting serum corticosterone levels in rats

Treatment	Dose µg/rat icv	Corticosterone µg/dl
Saline control	10 µl	6.3 ± 0.6
Verapamil	0.1	7.2 ± 1.2
Verapamil	1	9.1 ± 1.1
Verapamil	10	11.0 ± 3.3
	mg/kg ip	
Saline control	0.2 ml	11.0 ± 2.0
Verapamil	0.01	6.8 ± 1.4
Verapamil	0.1	9.2 ± 1.4
Verapamil	1	7.8 ± 1.7
Nifedipine	0.01	8.8 ± 1.0
Nifedipine	0.1	10.4 ± 1.9

Serum was obtained from trunk blood 1h after drug administration. Each value represents the mean ± SE of 7 rats.

2. Effect of calcium antagonists on phenylephrine-induced corticosterone response

Verapamil (0.1-1 μg) given icv significantly diminished, by 54%, the rise in the serum corticosterone levels elicited by phenylephrine, an α_1-adrenergic agonist, given by the same route 15 min later (*Fig. 1*). Also systemic administration of verapamil (0.1 mg/kg) produced a similar diminution, by 55%, of the phenylephrine-induced corticosterone response.

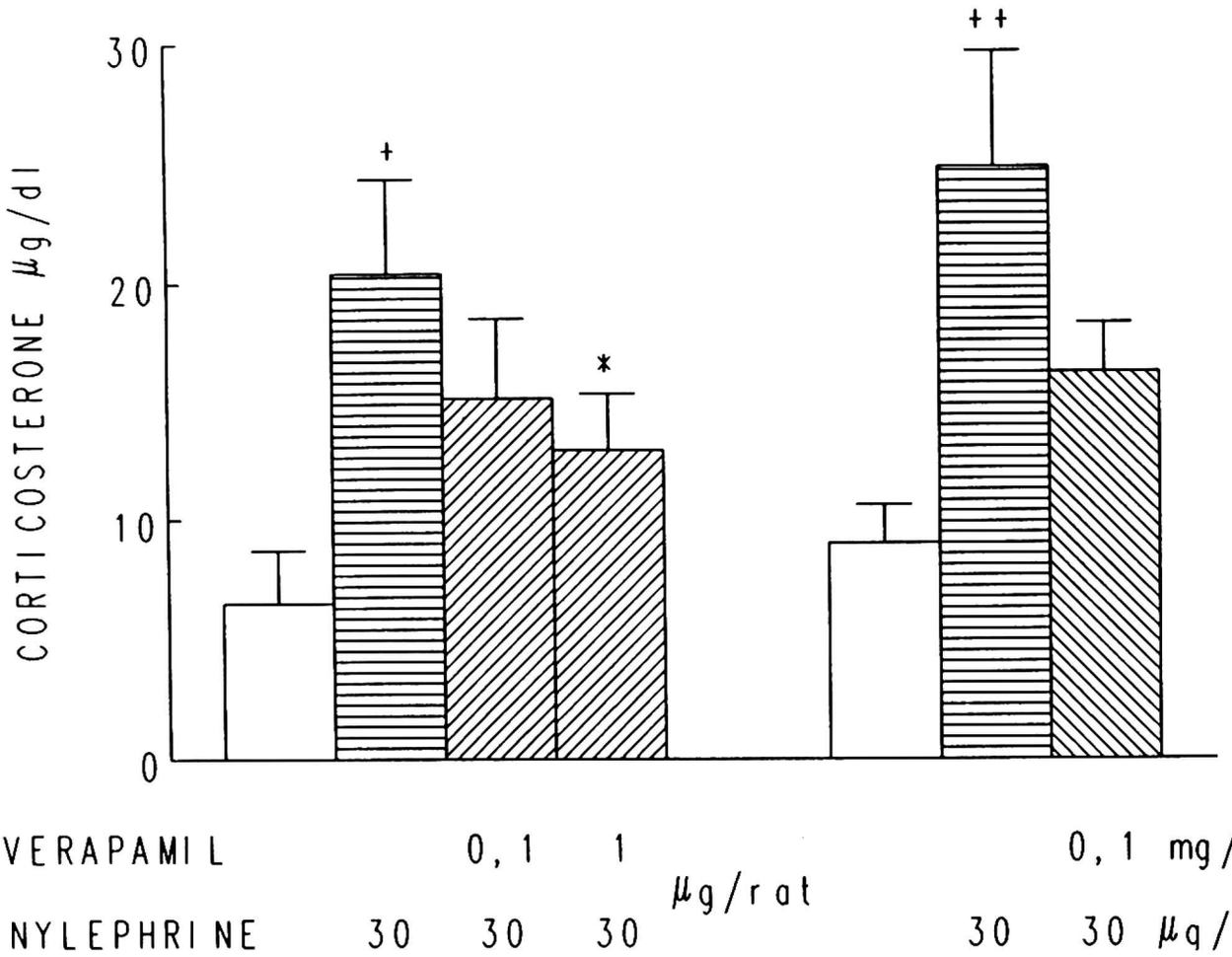

Fig. 1. Effect of verapamil on phenylephrine-induced corticosterone secretion. The drugs were given icv or ip, verapamil 15 min before phenylephrine and 1h later the rats were decapitated. Values represent the mean±SEM of 7 rats. + $p < 0.05$ vs. saline controls: * $p < 0.05$ vs. phenylephrine treated group.

Nifedipine (0.01 – 0.1 mg/kg), given systemically significantly decreased the corticosterone response to phenylephrine administered intraventricularly or intraperitoneally, by 64 and 74%, respectively (*Fig. 2*).

3. Effect of calcium antagonists on clonidine-induced corticosterone response

The significant rise in the serum corticosterone levels evoked by icv administration of clonidine, an α_2-adrenergic receptor agonist, was almost totally supressed, by 87%, by systemic pretreatment of rats with verapamil (*Fig. 3*). The above results suggest a considerable significance of calcium channels in the stimulating effect of clonidine on the corticosterone secretion.

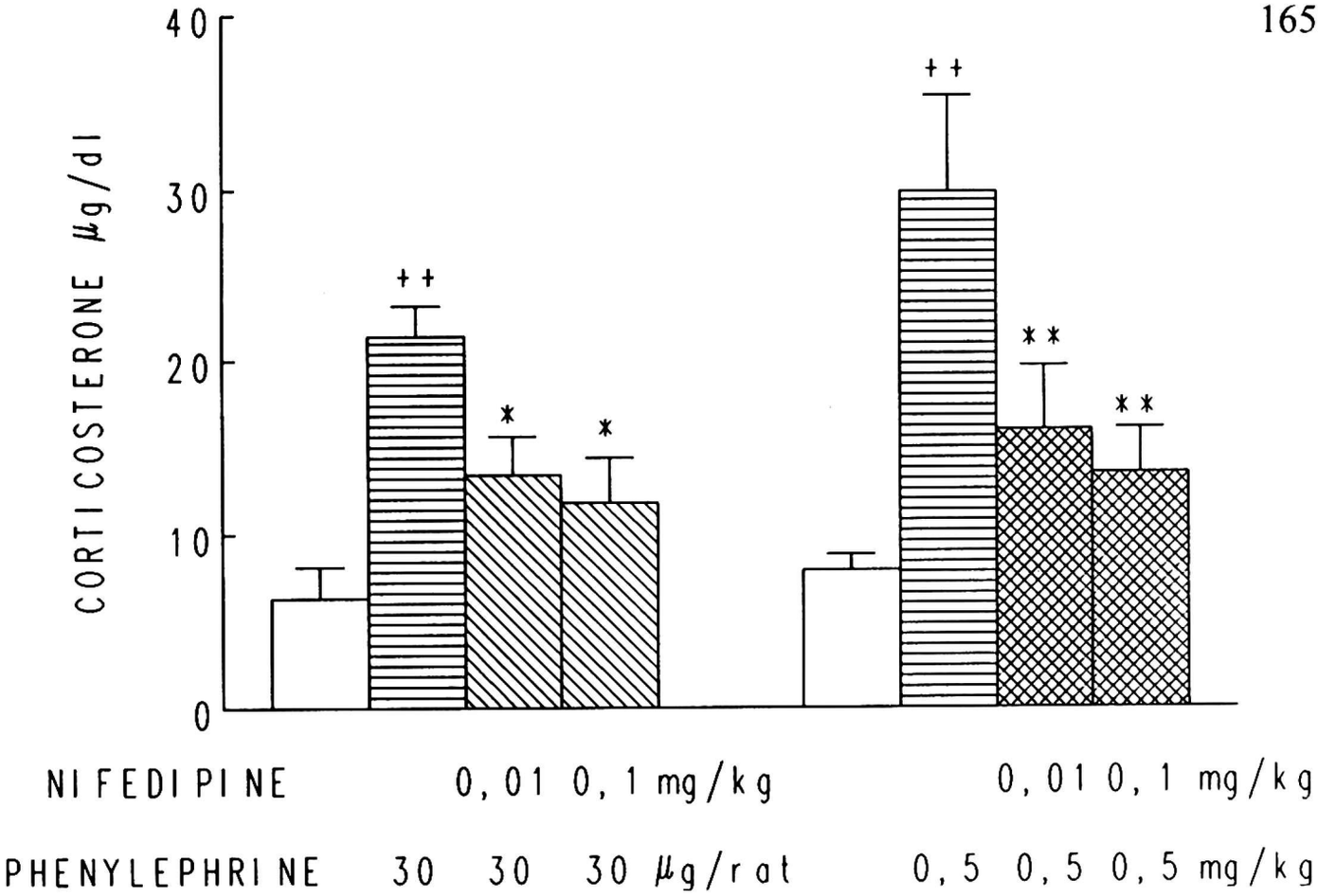

Fig. 2. Effect of nifedipine given ip on phenylephrine-induced corticosterone response. Values represent the mean ± SEM of 7 rats. ++ $p < 0.001$ vs. saline controls; * $p < 0.05$ and ** $p < 0.001$ vs phenylephrine treated group.

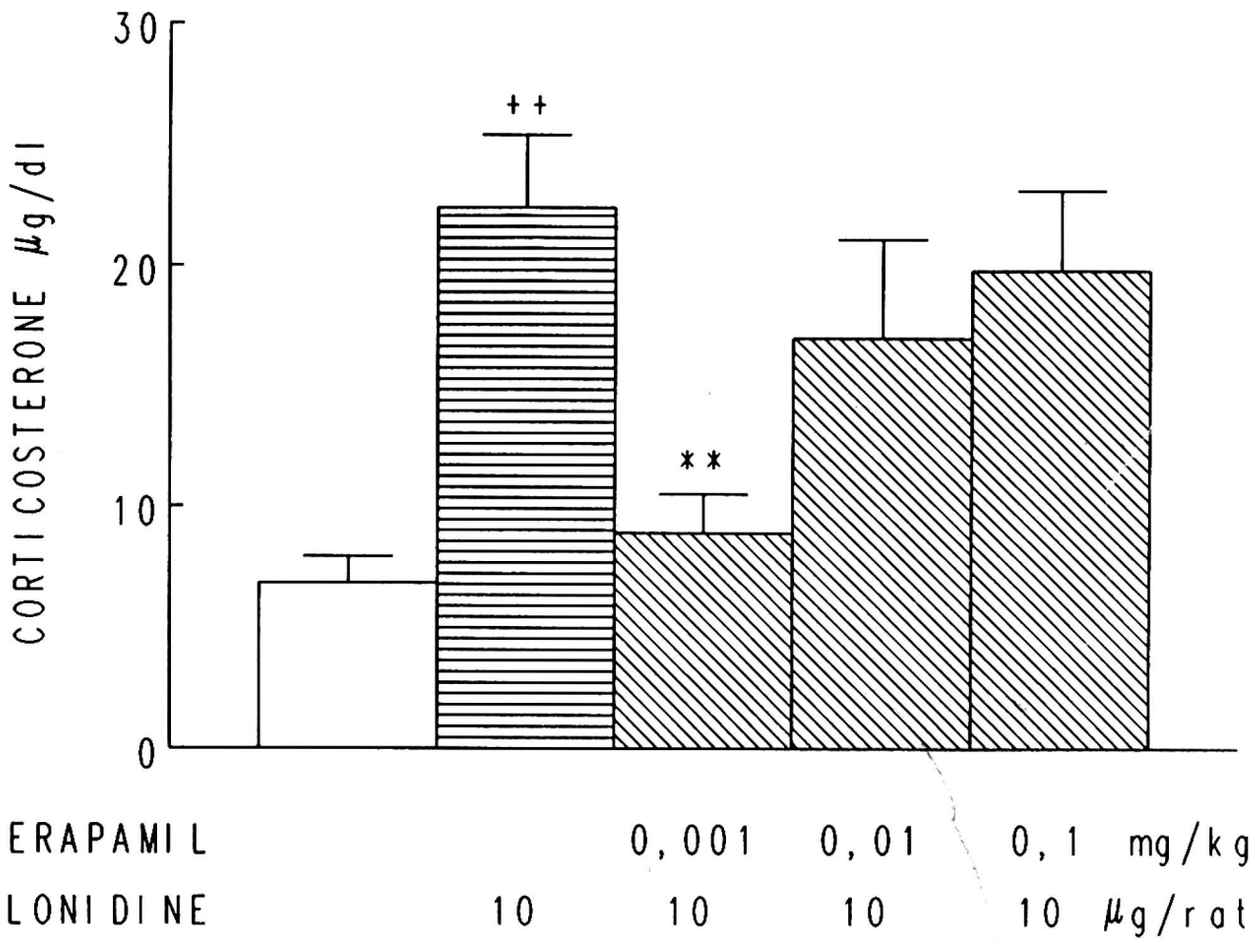

Fig. 3. Effect of verapamil given ip on clonidine-induced corticosterone secretion. Values represent the mean ± SEM of 7 rats. ++ $p < 0.001$ vs. saline controls; ** $p < 0.001$ vs. clonidine treated group.

4. Effect of calcium antagonists on isoproterenol-induced corticosterone response

The significant increase in corticosterone secretion, evoked by central administration of isoproterenol, a β adrenergic receptor agonist, was almost totally abolished by systemic pretreatment with verapamil (*Fig. 4*). Also nifedipine injected intraperitoneally impaired significantly, though not dramatically the rise in serum corticosterone levels evoked by systemic administration of isoproterenol (*Fig. 5*).

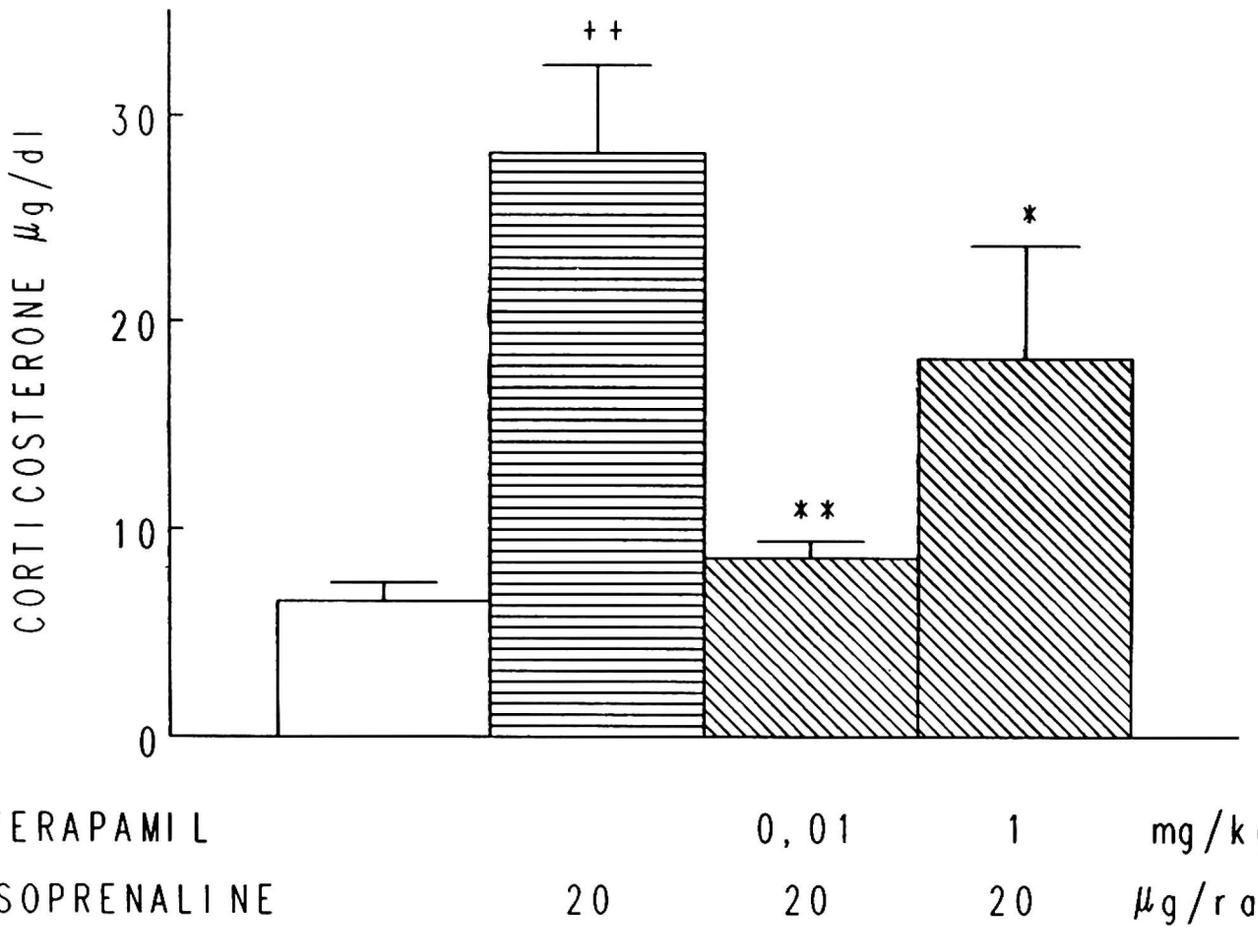

Fig. 4. Effect of verapamil given ip on isoprenaline-induced corticosterone response. Values represent the mean ± SEM of 7 rats. ++ $p < 0.001$ vs saline controls; * $p < 0.05$ and * $p < 0.001$ vs. isoprenaline treated group.

DISCUSSION

In the present experiment verapamil administered intracerebroventricularly or systemically decreased to the same extent, by 54%, the rise in the corticosterone secretion elicited by phenylephrine, an α_1-adrenergic receptor agonist. Our earlier experiment demonstrated that phenylephrine stimulated α_1-adrenergic receptors on both hypothalamic CRH-secreting neurons, as well as on anterior pituitary corticotrops which mediate corticosterone secretion (16). The present results suggest that verapamil may interfere with hypothalamic and pituitary calcium channels. It is known that α_1-adrenergic

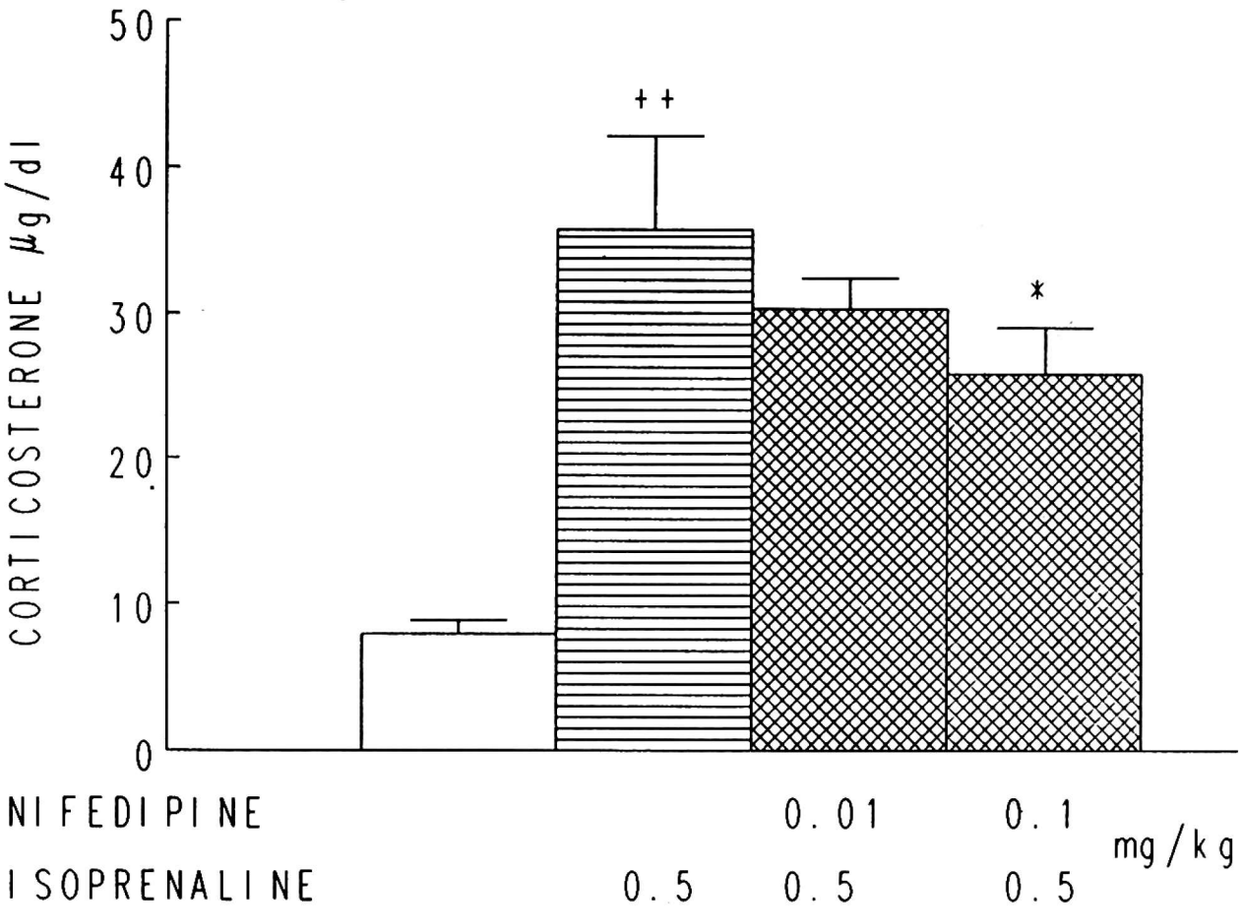

Fig. 5. Effect of nifedipine given ip on isoprenaline-induced corticosterone secretion. Values represent the mean ± SEM of 7 rats. ++ $p < 0.001$ vs. saline controls; * $p < 0.05$ vs. isoprenaline treated group.

receptors open calcium gates in the membrane by triggering phosphoinositide hydrolysis. Moreover, CRH is released from the hypothalamus in a Ca^{2+}-dependent manner (17), and anatomical evidence demonstrated a direct adrenergic neuronal control of CRH secretion. In the present experiment verapamil can inhibit the phenylephrine-induced corticosterone secretion by blocking calcium channels on the hypothalamic CRH-secreting neurons.

Some data suggest that after peripheral administration concentrations reached by verapamil in brain structures are considerably lower than in peripheral organs (18, 19). However, verapamil as highly lipophilic agent, probably crosses the blood-brain-barrier in an amount sufficient to affect the corticosterone secretion. After systemic injection verapamil may also block the ACTH release from corticotrops of the anterior hypophysis, which is not protected by the blood-brain-barrier. Another cause for reducing the phenylephrine-induced rise in corticosterone secretion is a possibility that verapamil binds to central α_1-adrenergic receptors that are involved in CRH/ACTH secretion, as has been demonstrated in myocardial adrenergic receptors (20-22). Therefore verapamil may be regarded as a weak antagonist of α_1-receptors, whereas this feature is hardly demonstrable in the case of nifedipine.

Nifedipine given systemically considerably reduced the corticosterone response to phenylephrine given either centrally or systemically. This inhibitory effect may be elicited by a decrease in ACTH release by CRH, as has been observed in isolated pituitary cells (10, 11), since nifedipine may block plasma membrane voltage-dependent-L-type calcium channels normally activated during CRH-evoked stimulation of pituitary corticotrops (8, 23).

The possibility of interaction of verapamil and nifedipine directly with the adrenal cortex has not been clarified. Although there is convincing evidence that stimulation of the adrenal cortex induces mobilization of Ca^{2+} from intracellular stores, it is not clear whether ACTH is capable of enhancing the Ca^{2+} influx into the adrenocortical cells via calcium channels (24).

Verapamil, given systemically, prevented almost totally the stimulating effect of clonidine, an α_2-adrenergic agonist, on corticosterone secretion. Our earlier experiments demonstrated that clonidine acted primarily at the hypothalamic but not pituitary or adrenal levels, since clonidine, when given systemically did not stimulate corticosterone secretion in rats (25). Therefore it is suggested that verapamil penetrates the hypothalamus from systemic circulation and inhibits the clonidine-induced CRH secretion. Verapamil may also reduce ACTH secretion by blocking calcium channels at the pituitary corticotrops. Since verapamil dramatically impairs, by 87%, the clonidine-induced corticosterone secretion, our results clearly show that calcium channels are of considerable significance in inducing stimulation of the hypothalamic-pituitary-adrenal axis by clonidine in rats.

Stimulation of α_2-adrenergic receptors is known to induce an influx of Ca^{2+} from the extracellular space across the membrane (26, 27). Alpha$_2$-receptors also mediate the noradrenaline-induced flux of calcium ions in rat peripheral neurons. However, the role of α_2-adrenergic receptors in the Ca^{2+} flux in the HPA system has not as yet been elucidated.

Verapamil given systemically was capable of abolishing almost completely the corticosterone response to isoproterenol, a β-adrenergic receptor agonist administered icv, also nifedipine significantly decreased the hormone response to that agonist given intraperitoneally.

It is well known that calcium ions are required to elicit β-adrenergic responses and isoproterenol stimulation of adenylate cyclase (28). Occupation of β-adrenergic receptors stimulates L-type calcium channels via cAMP-dependent phosphorylation, and by a direct coupling of the β-adrenergic agonist receptor complex to calcium channels via Gs protein (29, 30). Verapamil and nifedipine may interfere with both these components that are involved in the isoproterenol-induced corticosterone secretion.

The present data clearly demonstrate a significant role of calcium channels and calcium ions in the hypothalamic-pituitary-adrenocortical activity, stimulated by adrenergic receptor agonists. The inhibitory effect of verapamil

and nifedipine on this activity is mainly exerted at the hypothalamic and pituitary levels. Calcium blockers used for the treatment of circulatory and affective disorders may interfere with the function of the HPA, axis stimulated by the adrenergic system encountered during stress situations.

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