GALANIN STIMULATES CORTICOSTERONE SECRETION
BY ISOLATED RAT ADRENOCORTICAL CELLS

GIUSEPPINA MAZZOCCHI, LUDWIK K. MALENDOWICZ and GASTONE G. NUSSDORFER
Department of Anatomy, University of Padua, 35121 Padua, Italy

ABSTRACT
Galanin, a peptide isolated from pig intestine, dose-dependently stimulated basal corticosterone (B) secretion by isolated rat inner adrenocortical cells. The maximum effect was obtained at a peptide concentration of $10^{-7}$-$10^{-6}$ M. $10^{-6}$ M galanin did not enhance maximally ACTH-stimulated B production, though displaying additive secretagogue effect with submaximal effective concentrations of ACTH. Corticotropin-inhibiting peptide, a competitive inhibitor of ACTH, at a concentration ($10^{-6}$ M) which completely reversed the ACTH effects, did not affect the secretagogue action of $10^{-6}$ M galanin. These findings would indicate that galanin does not interfere with ACTH receptors, but probably activates, by binding to specific receptors, the second messenger system that mediates the glucocorticoid secretagogue action of ACTH. Since galanin is contained in chromaffin cells, the hypothesis is advanced that this peptide may be involved in the supposed paracrine control exerted by zona medullaris on the function of the adrenal cortex.

Galanin, a 28-amino acid peptide originally isolated from pig intestine (23), has been recently found to be very abundant in adrenal medulla of humans, cats and pigs (4-6, 13). Holst and associates (13) reported that galanin, which is co-stored with nor-epinephrine and released after splanchnic nerve stimulation, is able to evoke a steroidogenic secretory response by in situ perfused pig adrenals.

Here we report that rat galanin markedly enhances glucocorticoid production by dispersed inner adrenocortical cells of rats, without interfering with ACTH receptors.

MATERIALS AND METHODS
Adult male Wistar rats (300±30 g in body weight) were decapitated and their adrenal glands were promptly removed and freed of capsular fat. The glands were gently decapsulated and enucleated (to remove zona medullaris), and dispersed inner (zona fasciculata/reticularis) cells were obtained by collagenase-DNase I disaggregation (21). The viability of isolated cells was checked by the trypan blue exclusion test.

Dispersed cells were put in medium 199 (DIFCO, Detroit, U.S.A.) and potassium-free Krebs-Ringer bicarbonate buffer with 0.2% glucose (2:1 v/v) containing 5 g/l human serum albumin. Aliquots of cell suspensions (3×10$^5$ cells/ml) were incubated, in replicates of 6 each, as follows: i) increasing concentrations (from $10^{-10}$ to $10^{-5}$ M) of galanin (rat; Sigma, St. Louis, U.S.A.); ii) increasing concentrations (from $10^{-12}$ to $10^{-5}$ M) of human ACTH (Sigma) in the presence or absence of $10^{-6}$ M galanin; iii) $10^{-8}$ M ACTH or $10^{-6}$ M galanin in the presence or absence of $10^{-6}$ M corticotropin-inhibiting peptide (ACTH$_{1-38}$) (CIP; Peninsula, Merseyside, U.K.). The incubation was carried out for 90 min in a shaking bath at 37°C in an atmosphere of 95% O$_2$ and 5% CO$_2$.

Corticosterone (B) concentration in the incubation media was measured, after extraction and purification (20), by RIA (Cortex-RIA kit, Eurogenetix, Milan, Italy. Sensitivity: 25 pg/tube; cross-reactivity: B and cortisol 100%, 11-deoxycorticosterone and progesterone 2%, other steroids less than 0.001%). Intra- and inter-assay variations:
Fig. 1 Effect of galanin on the basal B production by isolated rat inner adrenocortical cells. Each point represents the mean ± SE of 6 separate estimates. +, P<0.05; *, P<0.01

Fig. 2 Effect of galanin (10^{-6} M) on ACTH-stimulated B production by isolated rat inner adrenocortical cells. Each bar represents the mean ± SE of 6 separate estimates. A, P<0.01 versus basal group; +, P<0.05; *, P<0.01 versus the respective none group.

Fig. 3 Effect of CIP (10^{-6} M) on basal, ACTH- and galanin-stimulated B production by isolated rat inner adrenocortical cells. Each bar represents the mean ± SE of 6 separate estimates. A, P<0.01 versus the basal none group; *, P<0.01 versus the respective none group.

7.5% and 9.1%.

Data were expressed as means ± SE, and their statistical comparison was done by ANOVA, followed by the Multiple Range Test of Duncan.

RESULTS AND DISCUSSION

Galanin dose-dependently raised basal B secretion by isolated rat inner adrenocortical cells (Fig. 1). The effect was already apparent at a galanin concentration of 10^{-8} M (44%), and reached its maximum at 10^{-7}-10^{-6} M (2-fold). This last finding accords well with that reported in vivo in the pig (13).

As expected, ACTH dose-dependently stimulated B production by dispersed cells (10^{-12} M: 2-fold; 10^{-10} M: 5-fold; 10^{-8} M: 9-fold); the maximal effective concentration of galanin (10^{-6} M) displayed additive secretagogue effects with the lower concentrations of ACTH (10^{-12} M: 86%, and 10^{-10} M:
54%), but it did not evoke any further enhancement in B secretion stimulated by 10^{-8} M ACTH (Fig. 2). Since this last concentration of ACTH is the maximal effective one in rats (19, 22), this result seems to suggest that galanin and ACTH share a common site of action in their secretagogue effects on inner adrenocortical cells.

The possibility that galanin may positively interfere with ACTH receptors of inner rat adrenocortical cells can be excluded by our experiment with CIP, a well-known competitive inhibitor of ACTH (15). In fact, 10^{-6} M CIP, which, as previously reported (2, 3), completely annulled the secretory response of isolated cells to 10^{-8} M ACTH (without changing basal B secretion), did not affect galanin (10^{-8} M)-evoked rise in B production (Fig. 3). These data would suggest that galanin, by binding to specific receptors, may activate the second messenger system that mediates the glucocorticoid secretagogue action of ACTH, thus being unable to magnify the maximal stimulatory effect of this agonist. We are currently investigating whether galanin is able to stimulate cyclic-AMP production in isolated rat adrenocortical cells.

The physiological meaning of our present results remains to be investigated. However, we want to recall that adrenal zona medullaris contains and releases, in addition to catecholamines, peptides, including enkephalins, vasoactive intestinal peptide, somatostatin, neuropeptide Y, substance P and calcitonin-gene related peptide (for review, see 14), which are reported to exert a direct regulatory action on adrenocortical secretory activity (1, 9, 11, 16-18). These data, together with the histologic demonstration of a strict interlacement between cortical and medullary tissues in the adrenal gland of rats (8, 10), allowed us to hypothesize that zona medullaris may exert a paracrine control on the adrenal cortex (7, 12). Thus, our present findings would suggest that galanin, high levels of which are present in chromaffin cells (4-6, 13), may be included in such a group of medullary adrenocorticotropic peptides.

Received 9 March 1992; and accepted 16 March 1992

REFERENCES
oidogenesis induced by ACTH$_{1-24}$, ACTH$_{1-10}$, ACTH$_{4-10}$ and ACTH$_{5-10}$. FEBS Lett. 19, 229–231


