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Effects of Somatostatin on Steroid Production by Adrenocortical Cells of the Domestic Turkey and Fowl

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ABSTRACT—The effects of somatostatin (SRIF) on *in vitro* avian adrenal steroid secretion have been investigated in the domestic turkey (*Meleagris gallopavo*) and fowl (*Gallus gallus domesticus*). SRIF did not affect either basal or ACTH-stimulated aldosterone and corticosterone production by dispersed turkey adrenocortical cells, but it concentration-dependently inhibited the secretory response to angiotensin II (ANG II). It is concluded that in the turkey, like in mammals, SRIF specifically interferes with the intracellular mechanisms transducing the ANG II secretagogue signal. Fowl adrenocortical cells did not display any secretory response to ANG II, and accordingly SRIF did not alter their basal and agonist-stimulated secretory activity.

INTRODUCTION

Many lines of evidence indicate that somatostatin (SRIF) plays an important physiological role in the modulation of adrenal mineralocorticoid secretion in mammals (for review, see Nussdorfer, 1996). However, the investigations dealing with the effect of SRIF on adrenal steroid secretion in lower vertebrates are very scarce and their findings are rather conflicting. Delarue *et al.* (1984) and Feuilloley *et al.* (1994) did not observe any effect of SRIF and urotensin II (a cyclic dodecapeptide exhibiting structural similarities with mammalian SRIF) on either basal or angiotensin II (ANG II)-stimulated aldosterone secretion by perifused frog adrenals. In contrast, Hanke and Kloas (1994) reported that urotensin II enhances adrenal steroid release in *Amphibia*. Studies on this matter are almost completely lacking in *Aves*: at present, only one investigation is available providing indirect evidence that SRIF may be involved in the negative regulation of adrenal steroid secretion in the rooster (Cheung *et al.*, 1988).

It, therefore, seemed worthwhile to examine the effect of SRIF on *in vitro* steroid secretion of adrenocortical cells of two avian species, the domestic fowl and turkey.

MATERIALS AND METHODS

Male domestic fowls (*Gallus gallus domesticus*) and turkeys (*Meleagris gallopavo*) of three months of age were obtained from the breeding facilities of our Agriculture School. The animals were decapitated, and their adrenal glands promptly removed. Glands were minced and stored in ice-cold Krebs-Ringer bicarbonate buffer with 0.2% glucose (KRBG), and dispersed cells were obtained by sequential collagenase digestion and mechanical disaggregation (Szalay, 1981). Dispersed cells were then collected and adrenocortical cells were separated from chromaffin cells on a preformed continuous density Percoll gradient, as described by Kocsis *et al.* (1994a). Dispersed cells obtained from adrenals of 2 animals were pooled to obtain a single cell suspension, and 6 cell preparations for each incubation experiment were employed.

Dispersed adrenocortical cells were put in a 2:1 mixture of Medium 199 (DIFCO, Detroit, MI) and KRBG, containing 5 mg/ml bovine serum albumin, and incubated (3×10^5 cells/ml) with the following peptides purchased by Sigma Chemical Co. (St. Louis, MO). (i) SRIF (from 10^{-10} to 10^{-5} M) in the presence or absence of 10^{-9} M ACTH or ANG II; and (ii) 10^{-9} M ANG II and 10^{-6} M cyclo(7-aminoheptanoyl-Phe-D-Trp-Lys-Thr[Bzl]) (SRIF-A), a specific competitive antagonist of SRIF receptors (Fries *et al.*, 1982), in the presence or absence of 10^{-7} M SRIF. The incubation was carried out in a shaking bath at 37°C for 90 min, in an atmosphere of 95% O_2 - 5% CO_2.

Aldosterone and corticosterone concentrations in the incubation media were measured, after extration and HPLC purification (Neri *et al.*, 1993), by specific RIA, as described previously (Malendowicz *et al.*, 1993). Intra- and interassay variations were: aldosterone, 7% and 8%; corticosterone, 6% and 9%, respectively.

Data were averaged per experimental group and SEM (n = 6) were calculated. Their statistical comparison was done by ANOVA followed by the Multiple Range Test of Duncan.

RESULTS

SRIF did not alter either basal or 10^{-9} M ACTH-stimulated aldosterone (Fig. 1) and corticosterone (Fig. 2) production by turkey interrenal cells. In contrast, it concentration-dependently inhibited ANG II (10^{-9} M)-enhanced hormonal secretion; minimal and maximal effective concentrations (10^{-8} M and 10^{-7} M, respectively) caused about 20% and 40% inhibitions (Figs. 1 and 2). The suppression of aldosterone and corticosterone responses to 10^{-9} M ANG II evoked by 10^{-7} M SRIF was abrogated by the simultaneous exposure to 10^{-6} M SRIF-A, which *per se* did not affect basal steroid secretion (Table 1).

SRIF was not found to induce significant changes in either basal or agonist-stimulated steroid secretion of dispersed fowl

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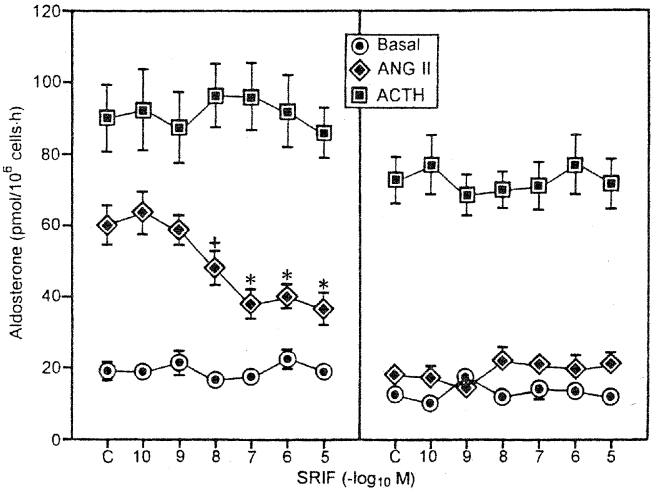

Fig. 1. Effect of SRIF on basal, and 10^{-9} M ACTH- or ANG II-stimulated aldosterone production by dispersed adrenocortical cells of turkeys (left panel) and fowls (right panel). Data are means ± SEM (n = 6). $^{+}P < 0.05$ and $^{*}P < 0.01$ from C.

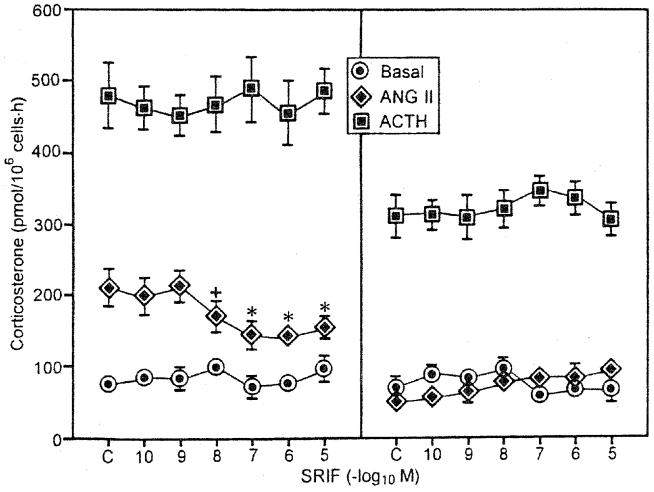

Fig. 2. Effect of SRIF on basal, and 10^{-9} ACTH- or ANG II-stimulated corticosterone production by dispersed adrenocortical cells of turkeys (left panel) and fowls (right panel). Data are means ± SEM (n = 6). $^{+}P < 0.05$ and $^{*}P < 0.01$ from C.

interrenal cells, which responded to ACTH, but not to ANG II (Figs. 1 and 2).

DISCUSSION

Our present findings clearly indicate that SRIF, acting via specific receptors, directly inhibits the steroidogenic response of turkey adrenocortical cells to ANG II. A large body of evidence indicates that in mammals SRIF specifically depresses secretion and growth of adrenal zona glomerulosa cells, by interfering with the main signaling mechanism transducing the ANG II secretagogue signal (i.e. the intracellular cascade following phospholipase C activation) (for review, see Nussdorfer, 1996). Hence, it may be conceived that the same mechanism underlies the inhibitory action of SRIF in the turkey, inasmuch as this peptide does not alter the secretory response of adrenocortical cells to ACTH, which is known to act by activating adenylate cyclase (for review, see Ganguly and Davis, 1994). However, it must be recalled that maximal effective concentrations of SRIF cause only a 40% inhibition of the secretory response to ANG II. This observation may be explained by taking into account that the mechanism underlying the secretagogue action of ANG II involves the activation of both phosphoinositide and tyrosine kinase pathways (Bodart *et al.*, 1995; Kapas *et al.*, 1995). Preliminary data (not shown), indicating that the selective tyrosine kinase inhibitor tyrphostin 23 (but not the phosphokinase C antagonist Ro31-8220) potentiates the effect of SRIF, strongly suggest that this peptide exclusively acts by blocking ANG II-stimulated phosphoinositide pathway in the turkey adrenocortical cells.

According to previous studies (Rosenberg *et al.*, 1988; Holmes and Cronshaw, 1993), dispersed fowl adrenocortical cells, though displaying a clear-cut secretory response to ACTH, are insensitive to ANG II. Probably, adrenocortical cells of this species, at variance with those of the turkey (Kocsis *et al.*, 1994b), are not provided with specific ANG II receptors; alternatively they could possess that subclass of ANG II receptors not coupled with a secretory response, which has been recently demonstrated to occur in the turkey adrenocortical cells (Kocsis *et al.*, 1995). Be that as it may, these findings easily explain why SRIF does not exert any

Table 1. Effect of 10^{-6} M SRIF-A on SRIF (10^{-7} M)-induced inhibition of secretory response of dispersed turkey adrenocortical cells to 10^{-9} M ANG II

Treatments		Aldosterone	Corticosterone
I.	Baseline	23.5 ± 2.1	94.5 ± 8.7
II.	SRIF-A	28.7 ± 3.2	99.7 ± 8.9
III.	ANG II	65.2 ± 6.2[a]	223.5 ± 30.1[a]
IV.	ANG II + SRIF-A	59.4 ± 6.5	231.4 ± 28.7
V.	ANG II + SRIF	40.8 ± 5.0[a,b]	148.7 ± 15.1[a,b]
VI.	ANG II + SRIF + SRIF-A	67.2 ± 7.1[a,c]	229.6 ± 27.5[a,c]

Data (pmol/10^6 cells • h) are means ± SEM (n = 6). [a]$P < 0.01$ from I; [b]$P < 0.01$ from III; [c]$P < 0.01$ from V.

direct inhibitory action on the secretion of fowl adrenocortical cells.

Cheung *et al.* (1988) have been reported that the *in vivo* administration of an anti-SRIF serum raises the basal blood level of corticosterone in the domestic chicken, thereby suggesting that endogenous SRIF plays a role in the regulation of adrenal steroidogenesis. Our present findings appear to conflict with this contention. A clue to reconcile this apparent discrepancy could be provided by the investigations showing that in mammals SRIF may regulate adrenal cortex indirectly by inhibiting the hypothalamo-pituitary CRH-ACTH system (Richardson and Schonbrunn, 1981; Reisine, 1985), as well as the release by adrenomedullary chromaffin cells of catecholamines (Moeller *et al.*, 1989), which are known to enhance basal adrenal steroid secretion (for review, see Nussdorfer, 1996). It remain to be settled whether these indirect mechanisms of action of SRIF are operative also in the *Aves*.

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