Effect of Synthetic Atrial Natriuretic Polypeptide on Hemorrhage-Induced Adrenocorticotropin Secretion of the Rat

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Abstract

The effect of synthetic alpha-human atrial natriuretic polypeptide (α -hANP) on the *in vivo* and *in vitro* release of ACTH and corticosterone was examined. In the *in vivo* study ACTH and corticosterone responses to rapid 2-ml/rat hemorrhage were measured in sixteen conscious rats after α -hANP administration. The hemorrhage increased plasma ACTH and corticosterone concentrations in the control group of rats ($p > 0.01$). ANP inhibited hemorrhage-induced ACTH secretion ($p < 0.05$), but the plasma corticosterone response was not affected. In the *in vitro* study a high concentration of ANP ($1 \mu\text{M}$) reduced basal corticosterone secretion from the isolated rat adrenal gland ($p < 0.05$), but the response to ACTH (10 ng/ml) and dibutyryl cyclic AMP (0.5 mM , 5.0 mM) was not affected. Our data suggest that ANP inhibits hemorrhage-induced ACTH secretion from the anterior pituitary but inhibits corticosterone secretion from the adrenal gland very weakly.

The presence of specific granules has been described in the mammalian heart atria, but their function has been unclear (Jamieson and Palade, 1964). Their number changes during sodium loading or depletion (de Bold, 1979). In 1981 de Bold *et al.* (1981) reported that crude extracts of rat atria injected intravenously produced a rapid and prominent, but transient, diuresis and natriuresis in anesthetized rats, and blood pressure was simultaneously decreased. Garcia *et al.* (1982) showed that natriuretic activity was closely related to the specific

granules of atria. Several groups of investigators independently purified and characterized novel peptides from rat atrial extracts (Flynn *et al.*, 1983; Atlas *et al.*, 1984; Currie *et al.*, 1984; Misono *et al.*, 1984; Napier *et al.*, 1984a; Seidah *et al.*, 1984). Kangawa and Matsuo (1984) successfully purified a human atrial natriuretic factor, determined its amino acid sequences, and named the peptide alpha-human atrial natriuretic polypeptide (α -hANP). These peptides caused prominent diuresis, natriuresis and vasodilation (Tang *et al.*, 1984; Seymour *et al.*, 1985). Recently, studies have shown that the synthetic atrial na-

triuretic polypeptide (ANP) inhibited glucocorticoid and mineralocorticoid secretions from the *in vitro* adrenal cortex (Chartier *et al.*, 1984; De Léan *et al.*, 1984a; Goodfriend *et al.*, 1984; Kudo and Baird, 1984), but the mechanism of ANP action on the adrenal corticosteroids is unknown.

To investigate the effect of synthetic α -hANP on the hypothalamo-pituitary-adrenal axis, we examined changes in ACTH and corticosterone concentrations in hemorrhage stress after α -hANP administration. An *in vitro* study was also performed using isolated rat adrenal blocks.

Materials and Methods

Cannula implantation

Twenty male Wistar rats (weighing 250–280 g) were anesthetized with sodium pentobarbital (45 mg per kg body weight, i. p.), and a silicon cannula (0.5 mm ID, 0.9 mm OD, Silastic® medical grade tubing, Dow Corning Corp., Michigan, U. S. A.) was inserted into the right jugular vein. The rats were caged individually and received food and water *ad libitum*. The details of this method were reported by Harms and Ojeda (1974). The day after cannula insertion, experiments were carried out in a quiet, comfortable room (0900–1200 h). One to 2 h prior to the experiment, a PE50 polyethylene tubing (Intramedic®, Clay Adams, New Jersey, U. S. A.) was connected to the cannula for blood collection. The collecting tubing and cannula were filled with saline containing heparin sodium (500 U/ml), and the end of the tubing was dangled from the cage. The rat was thus able to move freely.

Experiment-I-Effect on hemorrhage-induced response

Four of twenty rats was rejected for obstruction of the cannula. Sixteen rats were randomly divided into three groups. After 2 ml of blood was withdrawn from the cannula with a heparinized syringe, 0.5 ml of saline or synthetic α -hANP (3 μ g or 10 μ g per kg body weight) was injected intravenously via cannula. Ten and 30 min after injection, 0.5 ml of blood was collected and replaced immediately with the same amount

of saline. ACTH and corticosterone were measured from the blood samples.

Experiment II-In vitro study

Twenty rats weighing 350–400 g were decapitated. The bilateral adrenal glands were immediately collected. Each adrenal gland was cut into quarters and two pieces were placed into a polyethylene tube, and were incubated in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing glucose (0.2%), bovine serum albumin (0.25%), bacitracin 100 μ g/ml and ascorbic acid (1 mM). All tubes were placed in a water bath (37°C) and gently shaken (90 cycles per min) in a 95% O₂–5% CO₂ atmosphere. A series of two incubations was performed at 30-min intervals after preincubations. The medium was changed with each incubation, and test substances were added to the medium in the second incubation. The fluid was assayed for corticosterone concentration. Two separate experiments were carried out (1) The effect of synthetic α -hANP (10⁻⁶, 10⁻⁷, 10⁻⁸M) on the ACTH (10 ng/ml)-induced corticosterone secretion and (2) The effect of α -hANP (10⁻⁷M) on the dibutyryl cyclic-AMP (0.5, 5.0 mM)-induced corticosterone secretion. The net rate of corticosterone secretion into the medium was indicated by a ratio of the second to the first incubation.

Hormone assay

Blood was collected into chilled plastic tubes and centrifuged (1,200×g) at 4°C, and plasma samples were stored at -20°C until assay. The plasma ACTH concentration was measured with commercially available radioimmunoassay kits (CEA-IRE-Sorin, France). The plasma corticosterone concentration was also measured with commercially available cortisol radioimmunoassay kits (Daiichi Radioisotope Labs. Ltd., Tokyo). The availability of the kit for corticosterone assay has been described (Hattori *et al.*, 1986). Briefly, synthetic corticosterone was serially diluted with hormone free rat plasma to produce the standard curve. We used 50 μ l of samples for this assay. The cross reactivity of this anti-serum was 7.2% with corticosterone, 3.5% to 11-deoxy cortisol and less than 0.5% with other related hormones (Nakane *et al.*, 1980). As cortisol and 11-deoxy cortisol are thought to be negligible in the rat (Bush, 1951; Ward and Birmingham, 1960), values derived by this method were ascribed mostly to a true corticosterone

level. The correlation coefficient value obtained by the fluorescent method was $r=0.8554$ ($p<0.01$). The corticosterone concentration in the incubation medium was assayed by the fluorescent method (Guillemin *et al.*, 1959).

Synthetic α -hANP was purchased from the Peptide Institute (Osaka).

Statistical analysis

Values are presented as means $\pm$ SEM. The differences between the values were estimated by using Student's *t*-test.

Results

Effect on hemorrhage-induced hormone response

Plasma ACTH concentration continued to increase after hemorrhage, and rose to a ten-fold higher value than the basal level of the control group (Fig. 1a). ANP, however, attenuated the ACTH increase induced by hemorrhage (Fig. 1a). Plasma corticosterone responded to hemorrhage stress in all three groups ($p<0.05$, $p<0.01$). The increase seemed to be slightly greater in the control group, but there was no statistically significant difference among three groups (Fig. 1b).

Fig. 1a

Fig. 1b

Fig. 1. ACTH (a) and corticosterone (b) response to hemorrhage in rats treated with ANP. Results are expressed as mean $\pm$ SEM. * $p<0.05$, ** $p<0.01$ vs 0 time.

Effect on corticosterone secretion in vitro

A high concentration of ANP (10^{-6} M) decreased basal corticosterone secretion, whereas ACTH-induced secretion was not affected (Fig. 2a). ANP had no effect on the cyclic AMP-induced corticosterone secretion (Fig. 2b).

Fig. 2a

Fig. 2. Effect of ANP on corticosterone release from the isolated rat adrenal gland induced by ACTH (10 ng/ml) (a) and dibutyryl cyclic AMP (0.5 and 5.0 mM) (b). The number in each group is six ($n=6$). Results are expressed as mean $\pm$ SEM. The net rate of corticosterone secretion into the medium was indicated by a ratio of the second to the first incubation.

Discussion

It is interesting that the hemorrhage-induced ACTH secretion was inhibited by ANP. A similar effect was reported in vasopressin secretion stimulated by hemorrhage (Samson, 1985). Plotsky *et al.* (1985) reported that corticotropin-releasing factor (CRF) and vasopressin secretions from the median eminence were involved in hemorrhage-induced ACTH secretion. The inhibition of adenylate cyclase activity by synthetic ANF in the anterior and posterior pituitary was also demonstrated (Anand-

Srivastava *et al.* 1985b). Thus, ANP may inhibit ACTH secretion from the anterior pituitary via inhibition of adenylate cyclase activity in the pituitary or inhibition of CRF or vasopressin release from the median eminence.

The mechanism of ANP action on steroidogenesis is still unknown. Anand-Srivastava *et al.* (1985a, 1985b) demonstrated adenylate cyclase inhibition by synthetic ANP in various endocrine organs. Other investigators (Hirata *et al.*, 1984; Napier *et al.*, 1984; De Léan *et al.*, 1985a, Hori *et al.*, 1985; Ogura *et al.*, 1985; Shiffrin *et al.*, 1985) reported the presence of a specific ANP receptor in various organs. Goodfriend *et al.* (1984) postulated that ANP inhibited the early pathway of steroidogenesis in the adrenal cortex. Some reports have showed that ANP has an inhibitory effect on the basal aldosterone level and on the secretion from rat adrenal glomerulosa cells stimulated by ACTH or angiotensin II (Chartier *et al.*, 1984; Kudo and Baird, 1984). These results are not consistent with a study using crude atrial extracts (Atarashi *et al.*, 1984). Atrial extracts and synthetic ANP were reported to have no effect on the corticosterone release from rat zona fasciculata cells (Chartier *et al.*, 1984; Kudo and Baird, 1984). In studies using bovine zona fasciculata cells however, the results were different (De Léan *et al.*, 1984b; Goodfriend *et al.*, 1984; Kudo and Baird, 1984). De Léan *et al.* (1984b) demonstrated a specific receptor-mediated inhibition of synthetic ANP in cortisol secretion from bovine zona fasciculata cells. On the other hand, Kudo and Baird (1984) reported no effect on hormone secretion from rat and bovine fasciculata cells. In the present study, synthetic ANP showed only a weak inhibitory effect on basal corticosterone secretion in *in vitro* experiments. In addition, hormone secretion stimulated by ACTH or dibutyryl cyclic AMP was not affected by ANP. The dose

of ACTH which we used in the *in vitro* study was a submaximal one. Our data cannot rule out the possibility that the release of corticosterone induced by a lower dose of ACTH might be inhibited by ANP.

It has been known that hemorrhage is one of the strong stimuli to corticosteroid secretion. The release of ACTH is thought to be responsible for the adrenocortical response to hemorrhage. In the present study, however, there was no statistically significant difference in the corticosterone response to hemorrhage stress between control and ANP-injected groups although the ACTH response was strongly suppressed by ANP. We cannot explain this discrepancy. A significant difference might be found if blood collection was continued after 30 min of ANP administration. ACTH-independent mechanisms of the corticoid secretion may be involved in hemorrhage-induced response. Wood *et al.* (1982a) demonstrated the dissociation of ACTH and corticosteroid response to hemorrhage, and they presumed that this could be ascribed to an increase in the adrenal sensitivity to ACTH during hemorrhage (Wood *et al.*, 1982b). Direct neural control of the adrenocortical response is also postulated (Gann, 1979). In addition, it has been reported that there may be another ACTH-independent system in corticosteroid secretion (Graybeal *et al.*, 1985). It is possible that the ACTH-independent mechanism of corticosteroid secretion is modulated by ANP.

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