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PLASMA IMMUNOREACTIVE ATRIAL NATRIURETIC FACTOR (IR-ANF) INCREASES MARKEDLY AFTER α_2-ADRENERGIC STIMULATION WITH CLONIDINE IN NORMALLY-HYDRATED RATS

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Summary: Clonidine, an α_2-adrenergic agonist, induced a marked, dose-related increase of plasma IR-ANF in normally-hydrated rats. Maximal ANF release was observed at 10 min after injection of 50 µg clonidine, rising from 40.5 ± 4.6 pg/ml (x ± SEM) to 1064.4 ± 22.4 pg/ml. This effect on plasma IR-ANF was partially blocked by pretreatment with 0.8 mg naloxone, whereas synthetic Arg^8-vasopressin (AVP) did not inhibit clonidine's action. These findings indicate that increased ANF release may be involved in the mechanism of clonidine-induced diuresis. The clonidine's effect on ANF release may be mediated via activation of opioid receptors besides stimulation of α_2-adrenergic receptors.

Several studies (4, 9, 10, 13, 14) have reported that the α_2-adrenergic agonist, clonidine, produces diuresis in normally-hydrated rats, yet the mechanism underlying its diuretic action is not fully understood. Although some investigators (2, 9, 16) have suggested that this diuretic effect may be partially due to inhibition of arginine vasopressin (AVP) release, it is not always prevented by AVP, indicating that an unrelated mechanism may be involved (14).

There is some evidence of a relationship between the endogenous opiate and α_2-adrenergic systems (1, 3, 5, 12, 18). In order to elucidate the mechanisms of clonidine's diuretic action, α_2-adrenergic stimulation with clonidine and opiate blockade with naloxone were investigated in concert with IR-ANF release.

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MATERIAL AND METHODS

Animals

Female Sprague-Dawley rats weighing 200-250 g were housed 6 per cage and maintained on 12-h light-dark cycle. Food and water were available "ad libitum" for a few days before the experiments. In the first experiment, naloxone (Narcan-DuPont) was injected intravenously (i.v.) as a pretreatment, via the tail vein, at a dose level of 0.8 mg/rat. Clonidine (Catapress-Boehringer Ingelheim) was administered, also i.v., in a dose-response manner (1, 5, 10, 25, 50 μg). In all, seven nonanesthetized groups were studied, each containing 16 rats. Group I received 2 ml saline i.v. and, after 15 min, 300 μl saline i.v. Group II was given 2 ml saline i.v. and after 15 min 300 μl clonidine i.v. in a 1 μg-dose, group III, 5 μg clonidine, group IV 10 μg, group V 25 μg, and group VI 50 μg. Group VII was administered 2 ml of naloxone (0.8 mg) i.v. and, after 15 min 50 μg clonidine i.v.

In the second experiment, synthetic Arg^8-AVP (Peninsula Laboratories) was injected i.v. in a 5 ng dose with 50 μg clonidine. These drugs were dissolved in saline, which was also used for control purposes. The rats were decapitated 10 min after treatment.

In the third experiment, 50 μg clonidine was administered i.v. to 8 groups, each containing 15 animals, which were decapitated at 1, 2, 3, 4, 5, 10, 15, or 30 min after the injection. To measure urinary output, the rats were housed in metabolic cages after treatment, and their urine was funneled into graduated cylinders. Cummulative urine volumes were determined every 1h for 5 h after the administration of saline i.v. (group I), 50 μg clonidine i.v. (group II) or 50 μg clonidine + 0.8 mg naloxone i.v. (group III).

After decapitation, 2 ml of blood was collected in chilled tubes containing the following protease inhibitors: 1 mg of EDTA, 10 μl of 1 mM PMSF (phenylmethyl sulfonyl fluoride, Sigma No. P-7626), and 10 μl/0.5 mM pepstatin A (Sigma No. P-4265) per 1 ml of blood. The samples were centrifuged for 20 min. at 4,000 rpm at 4°C. The separated plasma was immediately extracted or stored at -70°F until assayed. Plasma IR-ANF was measured by radioimmunoassay with prior extraction on heat-activated glass, according to a previously-described method (6, 7). Briefly, 0.5 ml of a Vycor glass (Corning Glass Works #7930 140 mesh) suspension (50 mg activated glass powder in 1 ml distilled water) was added to 1 ml of rat plasma and agitated slowly in a cold room (4°C) for 30 min. After 3 min. of centrifugation at 3,000 rpm, the supernatant was aspirated and the glass powder was washed with 1.5 ml of de-ionized water. The absorbed ANF was eluted from the glass powder with 1 ml of 60% acetone in 0.05 MHCl. The acetone was evaporated in a nitrogen stream and the aqueous phase was lyophilized in a Speed-Vac. The residue was dissolved in 0.1 M phosphate buffer (pH 7.4) and ANF was determined by radioimmunoassay (6, 7). One-way analysis of variance (ANOVA), the Dunnet test and the unpaired t-test were used to determine the significance of the data.

RESULTS

Figure 1 illustrates that clonidine induced a dose-related increase of plasma IR-ANF with marked (20-fold) elevation after the highest dose (50 μg). This effect was partially blocked by pretreatment with naloxone (0.8 mg). The IR-ANF response to clonidine observed as early as 1 min after the injec-

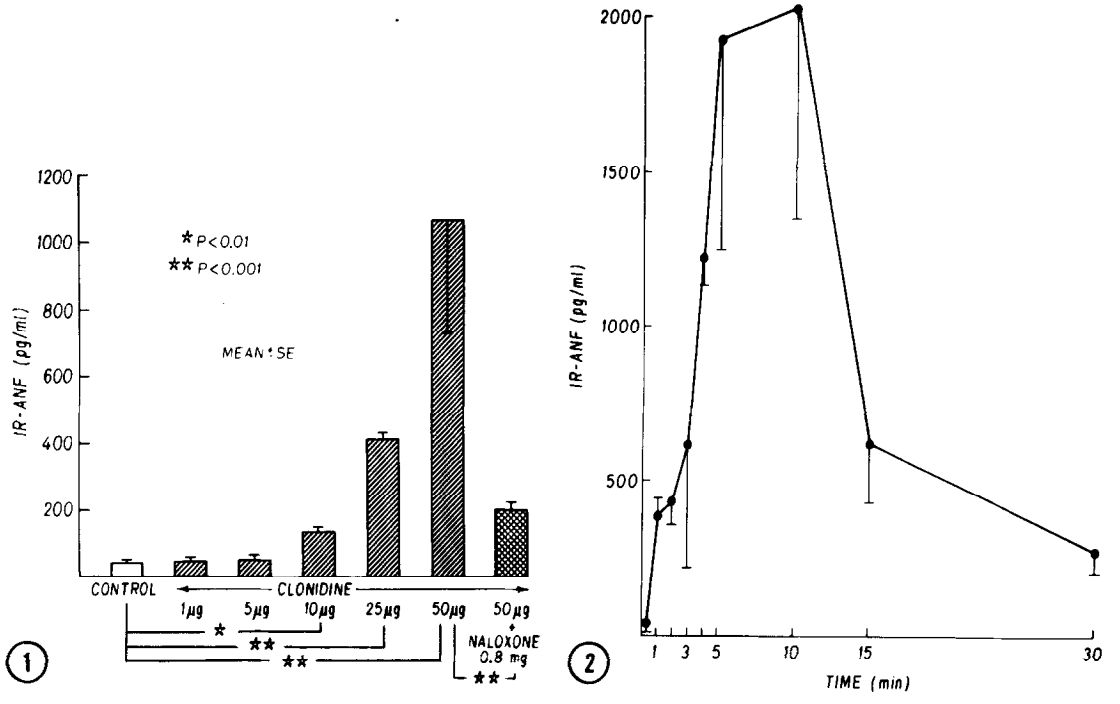

Figure 1. Effect of clonidine administered in a dose-dependent manner on plasma IR-ANF.

Figure 2. Plasma IR-ANF after administration of clonidine in a dose of 50 µg "time response".

tion was maximal at 10 min (Figure 2). Pretreatment with Arg^8-AVP did not inhibit the clonidine-induced release of ANF (Figure 3). The α_2-adrenergic agonist produced diuresis for 2 h while prior administration of naloxone significantly inhibited its diuretic effect within 1 h (Figure 4).

DISCUSSION

Our results demonstrate for the first time that clonidine elicits a marked increase of plasma IR-ANF in normally-hydrated, nonanesthetized rats. This increment of ANF correlates with the diuretic effect of the α_2-adrenergic agonist, indicating that enhanced ANF release may be involved in the mechanism of clonidine-evoked diuresis.

We also noted that the opiate antagonist naloxone, partially blocked clonidine's effect on plasma IR-ANF. Our previous studies have demonstrated that injection of morphine and other opiates led to a significant increase of

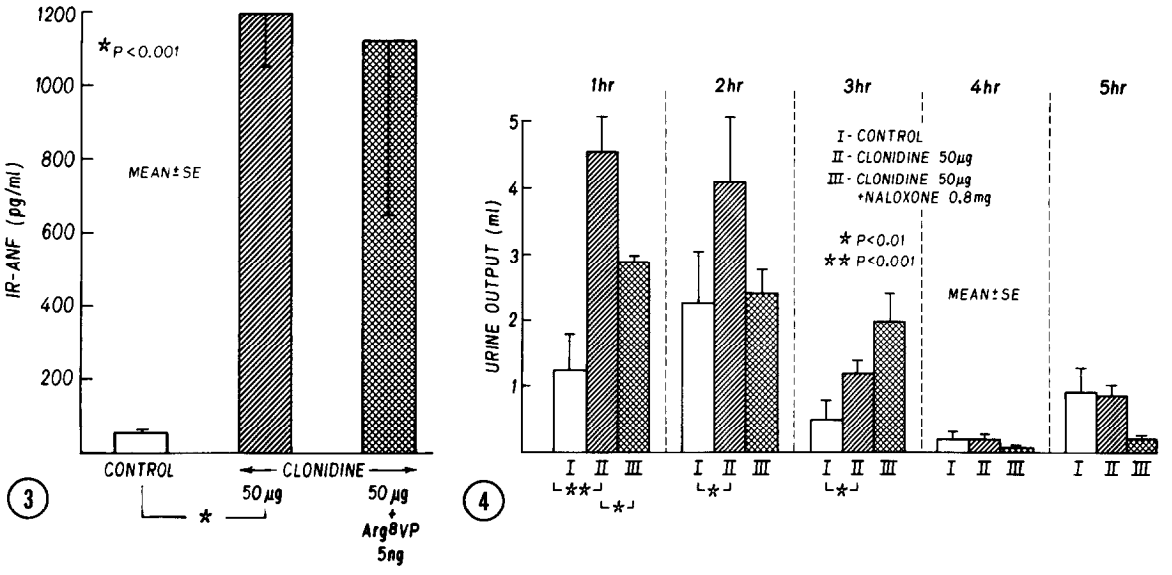

Figure 3. Effect of Arg^8 vasopressin pretreatment with clonidine on plasma IR-ANF.

Figure 4. Urine output after injection of clonidine in a dose of 50 µg.

plasma IR-ANF (8). It is possible that clonidine, besides stimulating α_2-adrenergic receptors, may activate opioid receptors and, consequently, may elevate ANF. There is abundant recent evidence of interactions between the endogenous opioid and α_2-adrenergic systems. Both α_2-adrenergic agonists and opiates depress the firing of noradrenergic (NE) neurons in the locus coeruleus (LC) (1, 12). Using an autoradiographic method, Young and Kuhar (17), have shown that opiate and α_2-adrenergic receptors are co-localized in the same cerebral areas. In some tissues, both types of receptors are localized presynaptically and inhibit the release of transmitters from the nerve terminals.

An interaction between the α_2-adrenergic antihypertensive drug clonidine and the opiate system of the brain has been reported recently (11). Independent of its influence on the function of neuronal opiate mechanisms in the brain, clonidine may exert some of its opiate-like effects by altering the release of β-endorphin from pituitary. Pettibone and Miller (15) have found that clonidine precipitates β-endorphin in the plasma of intact but not of hypophysectomized rats. It has been suggested that clonidine produces diure-

sis by inhibiting AVP release (2). However, studies by Leander et al. (14) have revealed that clonidine does not reduce plasma AVP levels in water deprived rats and our own results have indicated that synthetic AVP does not alter ANF release in response to clonidine. These findings confirm the hypothesis of Leander et al. (14) that the diuretic effect of clonidine is not related to AVP inhibition. In summary, clonidine evoked a marked increase of plasma IR-ANF in normally-hydrated rats, and this action was partially blocked by the opioid antagonist naloxone. The data suggest that ANF may be involved in the mechanism of diuresis caused by clonidine. The latter appears to enhance ANF release through stimulation of opiate and α_2-adrenergic receptors.

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