Kidney International, Vol. 46 (1994), pp. 1567–1569

Distribution of Fos in rat brain resulting from endogenously-generated angiotensin II

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Distribution of Fos in rat brain resulting from endogenously-generated angiotensin II. The beta adrenergic agonist isoproterenol has been used in these studies to elevate circulating levels of angiotensin II. Neurons in the brain responsive to the subcutaneous infusion of isoproterenol were identified using an antibody to Fos, the protein product of *c-fos* which is now used extensively as a marker of activated neurons. Fos-positive neurons were present in a range of specific forebrain and hind brain regions. Infusion of losartan (an angiotensin II type receptor antagonist) showed that neurons in the lamina terminalis were activated directly or indirectly by angiotensin II, whereas other neurons in the hypothalamus and brain stem were responsive as a consequence of the peripheral vasodilation caused by isoproterenol. The distribution of activated neurons in the lamina terminalis was consistent with that of neurons thought to be involved in water drinking.

The beta adrenergic agonist isoproterenol has been used extensively in studies of thirst mechanisms in the rat. Subcutaneous administration of isoproterenol results in a rapid water drinking response within minutes of its administration [1]. Isoproterenol treatment is also known to cause secretion of renin from the kidney by both a direct action on renal beta adrenergic receptors and as a secondary response to a reduction in arterial pressure associated with peripheral vasodilation [2, 3]. Consequently there is an increase in circulating levels of angiotensin II [4]. Isoproterenol-induced water drinking is abolished by prior nephrectomy [5, 6] or by treatment with an angiotensin antagonist or angiotensin converting enzyme inhibitors [6, 7], leading to the conclusion that isoproterenol-induced water drinking is, at least in part, mediated by the renin-angiotensin system. There is also evidence that mechanisms dependent on reduced baroreceptor input to be CNS may contribute to drinking [8].

Ablation of the anteroventral third ventricle wall (AV3V) of the rat brain results in a reduced dipsogenic response to isoproterenol [9] as does ablation of the subfornical organ [10]. In order to identify more precisely the regions of the CNS which are activated by isoproterenol treatment, we have mapped the distribution of neurons with elevated expression of the proto-oncogene c-*fos* following the administration of isoproterenol. It is now widely established that elevated levels of either the mRNA for c-*fos* or its protein product Fos can be correlated with increased neural activity in a range of systems [11–13]. Therefore the immunocytochemical location of Fos can be used as an anatomical marker of physiological activation.

In addition, the effect of losartan (an angiotensin II type I receptor antagonist), on isoproterenol-induced responses has been evaluated.

Methods

Animals

Male Sprague-Dawley rats weighing between 280 and 400 g were used in these experiments. They were housed in individual metabolism cages and were allowed access to pelleted food and water at all times other than during experiments. All experiments were carried out between 1200 hours and 1500 hours.

Experimental procedures

Rats were taken from their cages with a minimum of handling and injected subcutaneously with isoproterenol (Isuprel, Winthrop 50 μg/kg) either alone ($N = 5$) or in combination with an intraperitoneal injection of losartan (Dupont Merck, 100 mg/kg; $N = 4$). The administration of losartan preceded that of isoproterenol by 10 minutes. Some rats serving as controls received either a subcutaneous injection of isotonic (0.15 mol/liter) sodium chloride ($N = 5$) or were left untreated. Rats were allowed to survive in their cages for two hours after injections and were then anaesthetized with sodium thiopentone (100 mg/kg), and perfused transcardially with isotonic saline followed by approximately 300 ml of 4% paraformaldehyde in 0.1 mol/liter phosphate buffer.

Immunocytochemistry

Brains of prefused rats were removed, fixed a further 60 minutes in 4% paraformaldehyde then transferred to 0.1 mol/liter phosphate buffer containing 20% sucrose. Semi-serial, 40 μm sections were cut through preoptic, hypothalamic, midbrain and brainstem regions using a freezing microtome. Fos protein, elevated by the injection of agents outlined above, was detected using an antibody raised against a 14 amino acid sequence of human Fos (Ab-2, Oncogene Science, Manhasset, New York, USA). The antisera were subsequently localized using avidin-biotin-peroxidase techniques (Vector, Burlingane, California, USA) with the 3-3′ diaminobenzidine dihydrochloride chromagen enhanced with nickel ammonium sulphate to give a black reaction product.

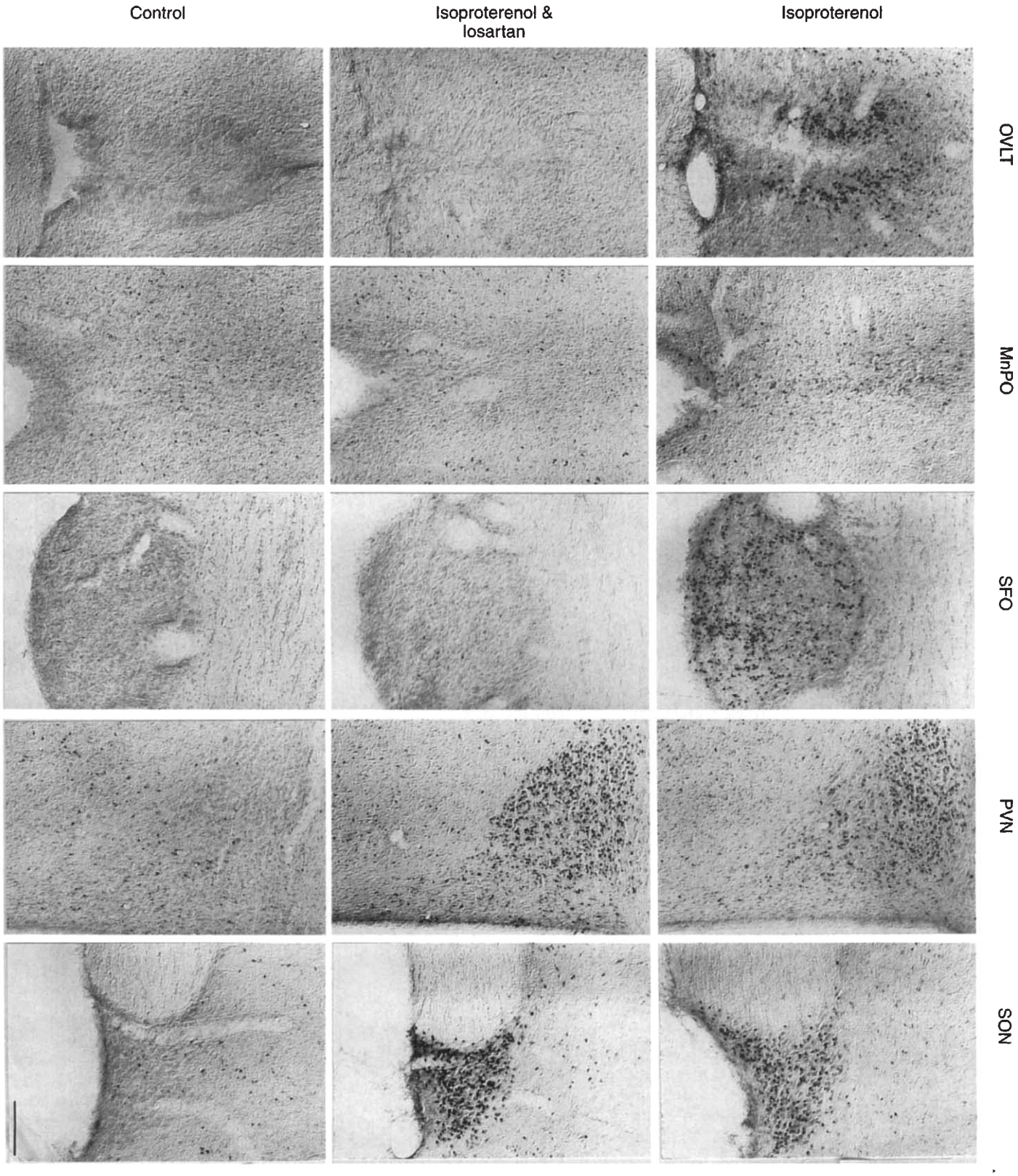

Fig. 1. *Series of bringhfield photomicrographs showing Fos-labeling (black dots) of neuronal cell nuclei.* The brain regions examined have been arranged in columns and treatments in rows. Areas of the brain include organum vasculosum lamina terminalis (OVLT); median preoptic nucleus (MnPO); subfornical organ (SFO); paraventricular nucleus (PVN); supraoptic nucleus (SON). III, third ventricle. Calibration bar = 200 μm.

Results

Isoproterenol treatment

Elevated levels of Fos protein were detected within neuronal cell nuclei in a range of areas which did not contain Fos-immunoreactivity in untreated rats or rats injected subcutaneously with isotonic saline.

Within the forebrain, the most conspiquous of these were the lamina terminalis, consisting of the subfornical organ, median preoptic nucleus and the organum vasculosum lamina terminals (OVLT), the supraoptic nucleus and both parvo- and magnocellular divisions of the hypothalamic paraventricular nucleus (Fig. 1). Other areas containing a density of Fos-immunoreactive nuclei above that in controls were the suprachiasmatic nucleus, bed nucleus of the stria terminalis, ventro lateral septum, central nucleus of the amygdala, as well as a range of thalamic nuclei. In the mid and hind brain intense Fos-positive nuclei were found in the locus coeruleus, nucleus tractus solitarii, area postrema and ventrolateral medulla.

Isoproterenol and losartan treatment

The intraperitoneal injection of losartan 10 minutes prior to isoproterenol treatment substantially reduced or abolished Fos labeling in the subfornical organ, median preoptic nucleus and OVLT (Fig. 1). Labeling in all other regions listed above remained high, notably the supraoptic and paraventricular hypothalamic nuclei (Fig. 1) and nucleus tractus solitarii, ventrolateral medula and locus coeruleus in the brain stem.

Discussion

The results of these experiments show that many areas of the brain known to be involved in the regulation of water intake (that is, lamina terminalis, bed nucleus of the stria terminalis), cardiovascular control (amygdala, area postrema, nucleus tractus solitarii, ventro lateral medulla, locus coeruleus) and neuroendocrine pathways (supra optic and paraventricular nuclei) are activated by isoproterenol treatment.

In regard to Fos-labeling in neurons of the forebrain, isoproterenol treatment causes renin secretion both as response to hypotension and as a direct effect of beta adrenergic stimulation of the kidney [5–8]. Fos immunoreactivity in the lamina terminalis is abolished by treatment with the angiotensin II type I receptor antagonist, losartan, indicating that this region is activated by circulating angiotensin II. Furthermore, intravenous infusion of angiotensin II has been shown previously to cause Fos-immunoreactivity in the lateral margins of the OVLT and throughout the subfornical organ [14]. A similar pattern was observed in the present experiments, consistent with the proposition that circulating angiotensin II directly stimulates neurons in these circumventricular organs, which are rich in high affinity angiotensin receptors [15, 16].

The Fos immunoreactivity found in midbrain and hindbrain regions, subserving autonomic activity, in all likelihood results from baroreceptor unloading secondary to the vasodilation and hypotensive effects of isoproterenol. Neurons in these areas will contribute to a reflex increase in sympathetic nerve activity.

These data highlight different populations of neurons activated by isoproterenol treatment. Those in the forebrain, which are stimulated by elevated levels of circulating angiotensin II, that correlate well with neurons thought to be involved with water drinking and the remainder, which are likely to be activated as a direct result of a reduction of blood pressure.

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