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Evidence of a Direct Action of Angiotensin II on Neurones in the Septum and in the Medial Preoptic Area[1]

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SIMONNET, G., B. BIOULAC, F. RODRIGUEZ AND J. D. VINCENT. *Evidence of a direct action of angiotensin II on neurones in the septum and in the medial preoptic area.* PHARMAC. BIOCHEM. BEHAV. 13(3) 359–363, 1980.—Angiotensin II (AII) was microiontophoretically applied on neurones located in the septum and the medial preoptic area (MPOA). All the septal neurones sensitive to AII (15/37) responded by an inhibition to the peptide application. Of 44 units tested in the MPOA 21 cells (48%) were sensitive to AII and responded either by an increase (11/21) or decrease (10/21) in their firing. The specificity of these responses were ascertained by simultaneous application of the antagonist Sar[1]-Ile[8]-Angiotensin II. These data suggest that Angiotensin II acts directly on neurones of the septum and medial preoptic area, structures implicated in the control of drinking behaviour.

Angiotensin II Iontophoresis Septum Medial preoptic area

ANGIOTENSIN II (AII) is now well established as a potent intracerebral dipsogen in every mammalian species so far examined (see general review by Phillips [20]). Recent studies have focused on the sites at which this peptide acts in the CNS. Periventricular structures appeared to be good candidates as the site for the mediation of the AII drinking response: subfornical organ [22], organum vasculosum of the lamina terminalis (OVLT) [16,19], anterior hypothalamus, septal and preoptic areas, [5], tissue surrounding the anterior third ventricle, [3,25] and mesencephalic central grey, [21]. Nevertheless, the mechanisms underlying the behavioural response are still unknown. Among the methods used to study the effect of a substance on the CNS, the technique of microiontophoresis is particularly suitable to elucidate the direct effect of AII on central neurones. Indeed, some previous reports have described an excitatory effect of AII when the peptide was microiontophoretically applied to supraoptic neurosecretory cells [17] and in: lateral hypothalamus, zone incerta and thalamus [27], subfornical organ [6,18], anterior hypothalamus [24] and more recently in the medial preoptic area [9] and the OVLT [7]. However, an inhibitory effect of AII has also been described in the lateral hypothalamus and thalamus [27]. The purpose of the present study was to examine the effects of the iontophoretic application of AII in two structures which have been previously implicated in the AII drinking response: septal and medial preoptic areas. Specificity of the AII response was systematically tested by the simultaneous microiontophoretic application of a potent competitive AII antagonist: Sar[1]-Ile[8] angiotensin II [4,11].

METHOD

Wistar rats of either sex (200–300 g) were anaesthetized with Ketamine (130–150 mg/kg/IP). A tracheotomy was performed and then, the animals were allowed to breath spontaneously. A glass multibarrel micropipette (7 barrels, tip diameter: 5–10 μm) was used; NaCl, 4 M was placed in the central barrel for recording (3–10 MΩ impedance) and one of the outer barrel. The rest contained one or other of the following drugs: angiotensin II (10^{-4} M, pH=4.5; Angiotensin human synthetic Beckman), Sar[1]-Ile[8]-angiotensin II (10^{-4} M, pH=4.5, Beckman), sodium L-glutamate (0.2 M, pH=7.5). Extracellular recordings were obtained from spontaneous active neurones and neurones in which firing was elicited by application of sodium L-glutamate.

Data were accepted only if they fulfilled the requirements stated by Zarzecki *et al.* [28] namely: (a) the increase or decrease in unit firing must exceed 50% of the baseline rate (threshold response) (b) the changes induced by the drug must be reversible and repeatable; (c) current of equal magnitude and polarity from the NaCl filled barrel must not duplicate the drug's effect. A response to AII was consid-

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TABLE 1

Sites	Recorded neurones	Neurones sensitive to AII	Neurones excited by AII	Neurones inhibited by AII
Septum	37	15	0	15
Medial preoptic area	44	21	11	10
Total	81	36	11	25

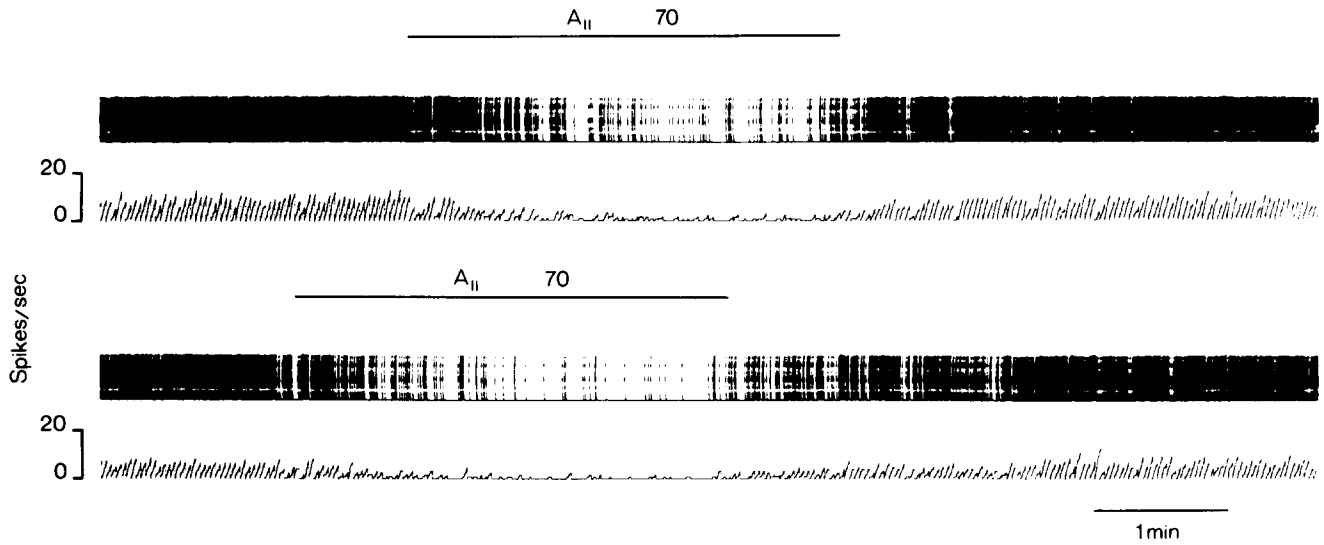

FIG. 1. Response of a single septal neurone to angiotensin II (AII); units (top) and integrated firing rate (lower trace); AII was applied with a cationic current (70 nA).

ered to be antagonised by the antagonist Sar¹-Ile⁸-AII if it was reduced by 50%.

The 7 barrel micropipette was placed on the brain surface 0.5–1 mm anterior to the bregma and 0.5–1 mm lateral to the midline. Recordings were made in an area 3.5–6.0 mm below the surface of the cortex for lateral septum and at a depth of 6.5–8 mm for medial preoptic area according the stereotaxic atlas [12]. At the end of each experiment, pontamine blue (a satured solution in the central barrel) was ejected with a cationic current (5 μA; 10 min) to permit subsequent location of the recording site.

RESULTS

General

The activity of 81 cells was recorded during the microiontophoretic study of the septal and medial preoptic areas. Thirty-six neurones (44%) were sensitive to the application of the AII (70 nA). The majority of sensitive neurones encountered were inhibited (25/36) (Table 1). This inhibitory effect could be demonstrated just as well on spontaneous firing units as those activated by glutamate. However, 52% of the sensitive units in the medial preoptic area were excited by application of AII. The AII-evoked effects (excitatory or inhibitory) were significantly reversed by the iontophoretic application of the antagonist Sar¹-Ile⁸-AII (70 nA) in 50% of sensitive units. When applied alone, the antagonist did not provoke any effect. Tachyphylaxis was not associated with the inhibitory or excitatory effect of AII even though the octapeptide was applied repeatedly to the same cell during periods in excess for 30 min. It was noteworthy that, in general, the microiontophoretic application of AII on sensitive units increased the amplitude of the recorded spike.

Depending upon Relationship to the Site Recording

Septum neurones recording studies. A total of 37 cells was recorded in the septal area. The effect of AII was to decrease the firing rate of sensitive units studied (15/15). The inhibitory effect of the peptide appeared with a latency of 10–30 sec after the beginning of the application of AII (70 nA). The maximal inhibition was generally observed 1 min after the beginning of AII ejection (Fig. 1). However, this inhibitory effect rapidly disappeared when the cationic current was switched off and the frequency of discharge returned to pretest levels. Of 15 cells that were inhibited by AII, the simultaneous iontophoretic application of Sar¹-Ile⁸-AII (70 nA) antagonised the response of 6 cells (40%).

Medial preoptic neurones recording studies. The action of AII was examined on 44 neurones located in the medial preoptic area. This structure contained a higher percentage of sensitive units (21/44) than in septal area. Furthermore, it was in this structure only that we found neurones excited by AII (11 of 21 sensitive cells). The regional specificity of this excitatory action could be clearly demonstrated with the

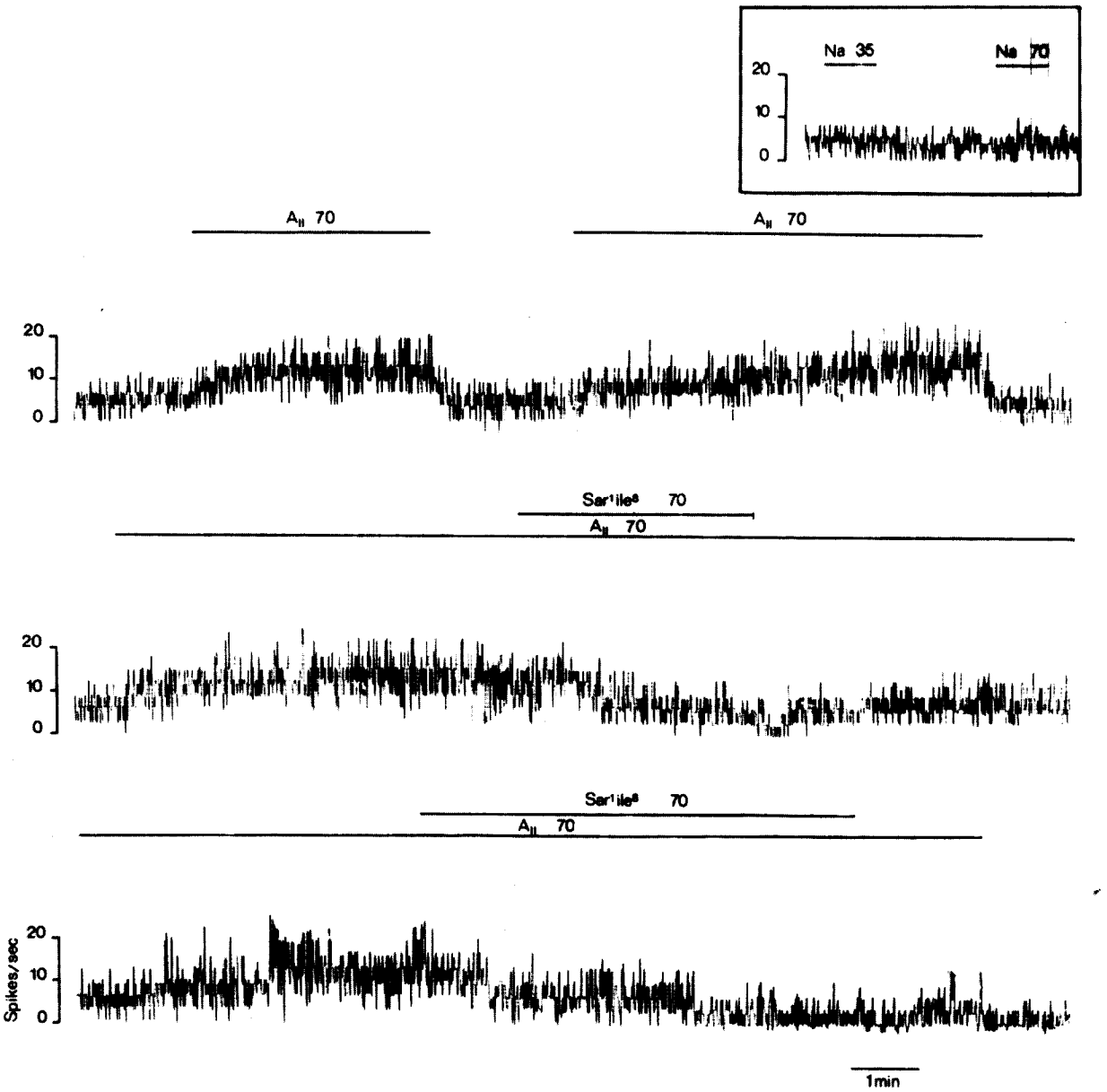

FIG. 2. Response of a single medial preoptic neurone to angiotensin II (AII); ratemeter output trace; AII was applied with cationic current (70 nA); Sar¹-Ile⁸-AII (Sar¹-Ile⁸) was ejected with a similar cationic current (70 nA); Inset: current control.

same electrode by recording from cells in the overlying septum during a single penetration. Illustration of this excitatory effect is shown in Fig. 2: the application of AII (70 nA) slowly increased the firing of neurones which rapidly returned to pretest levels when the cationic current was switched off. When AII was applied for a long period (see Fig. 2) the firing of cells remained to the new steady state. With the same conditions, the simultaneous application of Sar¹-Ile⁸-AII antagonised the excitatory or inhibitory effect and the firing of the cell return to the control level. The antagonism was observed in 12 of 21 sensitive units located in this area (57%). Antagonism of the AII effect by Sar¹-Ile⁸-AII was more pronounced of the AII excitatory effect (9/11) than on the inhibitory AII effect (3/10).

DISCUSSION

The present results have demonstrated that AII, when directly applied by microiontophoresis is able to affect the activity of neurones located in the medial preoptic area and septum. These are two regions where this peptide, when locally injected induces a drinking response.

The fact that it was possible to reverse the peptide effect by the simultaneous application of Sar¹-Ile⁸-AII, a powerful antagonist of AII [4,11] is in favour of the existence of specific receptors for AII in these two central structures. This idea agrees with certain biochemical and histological studies: (1) significant amounts of AII were "specifically" bound in all areas bordering on the ventricles: lateral septum, preoptic area, anterior hypothalamus and medial midbrain [23]; (2) scattered AII containing nerve terminals have been found in the preoptic area and the septal region [8].

From our results, the mechanism of action of AII on the neurones of the MPOA may be discussed. Among the AII-sensitive neurones in this area, a consistent number of cells

(52% of AII-sensitive neurones) responded by an excitation to the peptide application. Similar results have been reported by Gronan and York [9]. This AII induced activation, reversed in 85% of cases by Sar^1-Ile^8-AII added to the previous behavioural and biochemical data strongly suggests that MPOA is a central target for AII.

Thus, AII receptors in MPOA may be the origin of descending pathways involved directly in the drinking response. These pathways, described by Swanson, Kucharczyk and Mogenson [26] include the lateral and posterior hypothalamic areas, the region of the ventromedial nucleus, the mammillary body, the ventral tegmental area and the midbrain central gray. This hypothesis is reinforced by following observations: (1) AII applied to the MPOA changes the firing rate of neurones located in the lateral hypothalamus and the ventral tegmental areas [13]; (2) lesions of these two structures abolish the drinking response to AII injected in the MPOA without affecting the drinking response induced by administration of the peptide in the subfornical organ (SFO) or anterior third ventricle [14].

On the other hand, the univocal inhibitory effect of AII on the AII-sensitive neurones in the septal area agrees with the hypothesis of the inhibitory function of septum in the thirst mechanism: (1) septal lesion often produces hyperdipsia [1,10]; (2) electrical stimulation of the septum has been reported to specifically suppress thirst in which the renin-angiotensin participates [2]. So, AII may play a role in the control of the firing action of septal cells involved in water ingestion.

In conclusion, it can be put forward that the central mechanism by which AII triggers the drinking response may have two components. The first component may be due to diffusion of the peripheral peptide into the circumventricular structures such as SFO and OVLT. The second component may involve a direct action of AII on structures such as the MPOA which is closely located to efferents pathways mediating the drinking response, or the septum which plays a modulator role on the building-up of drinking behaviour via an inhibitory function.

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