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Effects of Angiotensin II on Central Neurons[1]

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WAYNER, M. J., T. ONO AND D. NOLLEY. *Effects of angiotensin II on central neurons.* PHARMAC. BIOCHEM. BEHAV. 1 (6) 679 691, 1973.–Effects of angiotensin II adminstered intravenously and by means of electrophoresis through multibarrel micropipette electrodes on the frequency of extracellularly recorded action potentials of brain cells were determined. A total of 293 neurons in seven different parts of the brains of male and female hooded rats anesthetized with urethan or a mixture of urethan and chloralose were tested. Results indicate that angiotensin affects many different cells within the lateral hypothalmus, zona incerta, ventromedial and dorsomedial hypothalamic nuclei, dentate gyrus and thalamus. The cells of the LH and zona incerta are the most sensitive to angiotensin and two types of lateral hypothalamic neurons were found which were affected differently by angiotensin and stimulation of the basolateral amygdaloid nucleus. Some of the Na sensitive neurons, all of which were sensitive to angiotensin, displayed a very pronounced potentiation of discharge frequency when angiotensin and Na were administered simultaneously. Angiotensin II might therefore influence drinking because of a relatively low threshold effect on hypothalamic neurons.

Angiotensin II   Drinking   Hypothalamus   Zona incerta   Thalamus   Dorsomedial hypothalamic nucleus
Ventromedial hypothalamic nucleus   Dentate gyrus   Microelectrophoresis

ANGIOTENSIN appears to be involved in body fluid regulation by stimulating the release of aldosterone and antidiuretic hormone [8] and a possible direct action on hypothalamic neurons implicated in the control of drinking [7]. The results which indicate that the preoptic region, anterior hypothalamus, lateral hypothalamus, and septum are differentially sensitive to angiotensin seem to be confounded by the probable spread of the administered fluid along the shaft of the indwelling chronic cannulae into the ventricles with possible action at some other tissue sites [13,20]. Since there is no evidence that angiotensin crosses the blood brain barrier [17] and intraventricular administration elicits drinking [6], an indirect route to central neurons via the choroid plexuses, cerebrospinal fluid and ventricles particularly into the walls of the third ventricle [23] seems very likely. In addition, both angiotensin and carbachol when injected into the midline region of the rat brain produce drinking [21]. These data cast serious doubts on the alleged functional significance of the renin-angiotensin system in the elicitation of drinking and raise the important question of specific sensitivity of central neurons to angiotensin. Three brief reports have appeared recently in which the sensitivity of hypothalamic neurons to angiotensin was determined by more direct means [16, 19, 24]. The purpose of the present study was to examine in greater detail the effects of valine^5-angiotensin II on the discharge frequency of hypothalamic and other central neurons of the rat when applied intravenously and by means of microelectrophoresis. Results indicate that angiotensin affects many different cells within the lateral hypothalamus (LH), zona incerta, ventromedial and dorsomedial hypothalamic nuclei, dentate gyrus and thalamus. The cells of the LH and zona incerta are the most sensitive to angiotensin and two types of LH neurons were found which were affected differently by angiotensin and basolateral amygdala stimulation. Some of the Na sensitive neurons, all of which were sensitive to angiotensin, displayed a very pronounced potentiation of discharge frequency when angiotensin and Na were administered simultaneously. Angiotensin might influence drinking because of a specific effect on some ionic mechanism in Na sensitive hypothalamic neurons.

METHOD

Animals

Experiments were performed on male and female hooded rats, 245–290 g in weight, selected from our colony.

Procedures

In the experiments employing intravenous administration techniques, animals were anesthetized with

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50 mg/kg of urethan. When electrophorectic ejection of angiotensin II and Na was used, animals were anesthetized with a mixture of chloralose, 50 mg/kg, and urethan, 0.5 g/kg, administered intraperitoneally. Subsequent maintenance doses of chloralose, 10–20 mg/kg, and urethan, 0.1–0.2 g/kg, were injected when necessary. The intravenous doses of angiotensin II, CIBA HYPERTENSIN, were administered as a single injection of 5 μg in 0.05 ml of distilled water at the rate of 0.54 ml/min. The dose was calculated from the earlier work of others [9]. The NaCl solution was administered in the same way, a standard volume of 0.05 ml of 15% NaCl.

The coordinates of recording and stimulating sites were taken from the de Groot atlas [11]. Three barrel recording electrode arrays were inserted into the LH according to the following coordinates: A 5.8, L 1.9, H −2.9, with 10–15° from the vertical line. Briefly, three barrel glass capillary microelectrode arrays were used. Extracellular single unit discharges were recorded through one barrel filled with 4 M/l NaCl, resistance varied from 5–25 MΩ. Action potentials were amplified, monitored visually, stored on magnetic tape, and analyzed by conventional means. The other two barrels were filled with angiotensin II, CIBA 83% valine[5]-angiotensin II and 17% ammonium acetate, 12–60 mg/ml in distilled water with a final pH of 5.0; and monosodium-l-glutamate, 2 M/l, pH of 8.0 (NaOH). Both substances were ejected in the vicinity of the tip mainly by outward currents supplied by a constant current source [25]. The resistances of these two barrels varied from 5–80 MΩ. The overall tip diameter of the three barreled array varied form 3–6 μ. Possible direct electrical effects of the microelectrophoretic currents were evaluated on the basis of previously published criteria [5] and unreliable data were discarded. Tests performed with ammonium acetate alone in distilled water were negative and indicate that the effects reported here can be atrributed to the angiotensin.

Bipolar concentric stimulating electrodes were placed in the lateral part of the basolateral amygdaloid nucleus (ABL) according to the following coordinates: A 4.6, L 4.8, H −3.6. The outer pole was made of 25 gauge, 0.4 mm O.D., stainless steel tubing insulated with varnish and the inner electrode was made of enamel coated stainless steel wire. Tip separation was 0.2–0.4 mm and the DC resistance was 20–60 kΩ. Rectangular stimulating pulses 0.05–0.4 mA, 0.01 or 0.1 msec duration were delivered through a stimulus isolation unit. The inner electrode was negative. In general the remainder of the procedures were as described previously [26].

The final electrode tip position within the brain for each experiment was determined by perfusing the animal with the electrode shaft in place. After fixation the electrode was removed and the brain was sectioned at 40 μ, stained with cresyl violet, and examined by means of a dissecting microscope.

RESULTS

Effects of Intravenous Administration of Angiotensin II and NaCl

The data on 79 cells from 7 different brain sites are summarized in Table 1. The data on these 79 cells were selected from a total of 326 cells and represent clear and reliable results. As reported previously [24], two different types of neuron were found in the LH; those definitely within the medial forebrain bundle (LH-MFB), and another variety located about 0.5 mm more ventral and more medial (LH) from the center of the LH. The differences between these two types of cell will be described in more detail when the data obtained by the electrophoretic application of angiotensin is discussed. In general the LH cells have relatively low spontaneous discharge frequencies at the time they are encountered and for this reason are usually difficult to locate. The range in discharge frequency for the 11 LH cells in Table 1 is 0–25 per sec with a mean of 3.1 per sec and a standard deviation of 3.9 per sec. Of the 11 tested with angiotensin, 3 increased (I) and 5 decreased (D) in discharge rate and 3 were not affected (NE). The increase in one cell is illustrated in Fig. 1 where the discharge frequency in spikes per sec is plotted as a function of time. The intraveneous administration of angiotensin and ringer's solution is indicated by the downward vertical arrows. The increase due to angiotensin is obvious. A cell which decreased in discharge frequency in response to angiotensin is illustrated in Fig. 2. Both cells were affected in the same way by the intravenous administration of 0.05 ml of 15% NaCl, not illustrated. Of the 3 LH cells which increased, 2 were tested with Na and increased. Three of the 5 LH cells which decreased were tested with Na and they decreased. One of the 3 LH cells which was not affected by angiotensin was tested with Na and was not affected.

TABLE 1

A SUMMARY OF THE EFFECTS OF INTRAVENOUS ANGIOTENSIN II AND Na ON THE NEURONS OF SEVEN DIFFERENT BRAIN SITES

	ANGIOTENSIN II				Na			
Site	N	I	D	NE	N	I	D	NE
LH	11	3	5	3	2/3	2		
					3/5		3	
					1/3			1
LH-MFB	20	9	0	11	9/20	9		
					11/20			11
Zona Incerta	16	6	4	6	4/6	4		
					1/4		1	
					6/6			6
VMH	10	3	0	7	1/3	1		
					2/7			2
DMH	7	4	0	3	1/4	1		
					1/3			1
Thalamus	6	2	0	4	3/4		2	1
Dentate Gyrus	9	4	2	3	1/4	1		
					1/2		1	

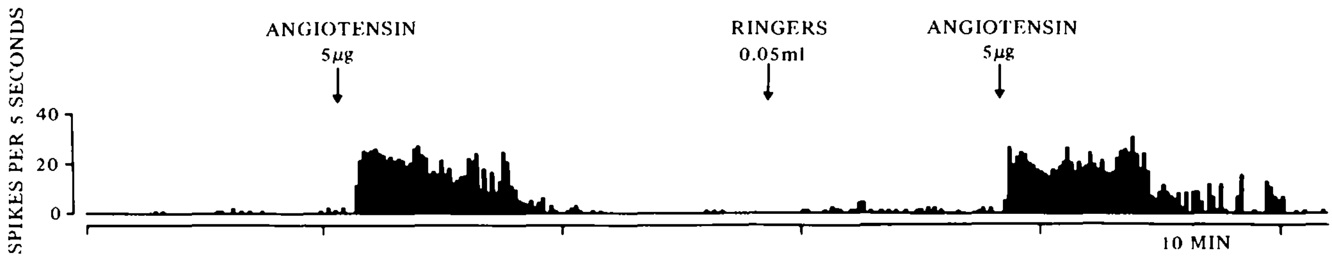

FIG. 1. An X-Y plot of the discharge frequency of an LH neuron illustrating the effects of intravenous administration of 5 μg of angiotensin and an equal volume of ringers solution. Downward arrows indicate the beginning of the infusions. Angiotensin increased the discharge frequency.

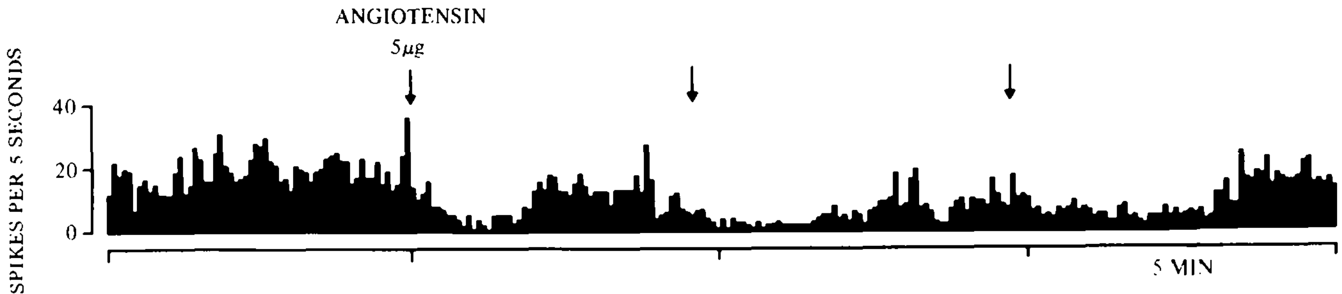

FIG. 2. An X-Y plot of the discharge frequency of an LH neuron illustrating a decrease in frequency produced by intravenous administration of 5 μg angiotensin applied at each downward pointing arrow.

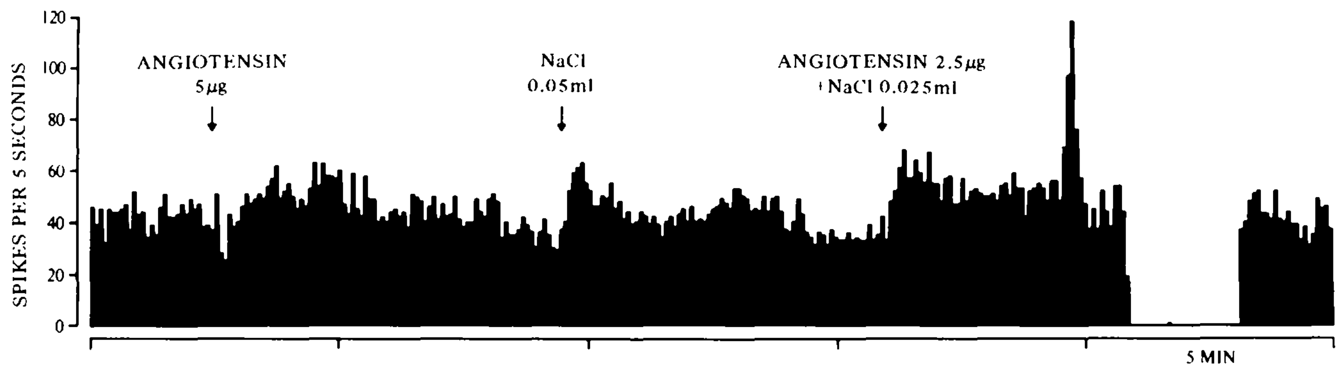

FIG. 3. An X-Y plot of the discharge frequency in an LH-MFB neuron illustrating the increase due to intravenous administration of 5 μg of angiotensin and then the potentiation produced by the intravenous administration of an equal fluid volume of 2.5 μg of angiotensin plus 0.025 ml of 15% NaCl. Downward arrows indicate when the infusion pump was turned on.

Several of the LH and LH-MFB cells which respond to both angiotensin and Na alone displayed a large multiplicative increase in discharge frequency in response to mixing both solutions in a single administration. The effect is illustrated in the 2 LH-MFB cells of Figs. 3 and 4. In the combined treatment only half of each previously administered dose was mixed together and consequently the volume injected remained the same as for a single administration. An obvious postexcitatory depression was observed in some neurons. The magnitude of the changes are also different for the two cells.

Twenty LH-MFB cells were tested with angiotensin and 9 increased in frequency, none decreased, and 11 were not affected. These data are summarized in Table 1. The 9 cells which increased were also increased by Na and the other 11 were also not affected by Na. In the zona incerta 16 cells were examined and 6 increased in response to angiotensin, 4 decreased, and 6 were not affected. Four of those which increased were tested with Na and all 4 increased. One of the 4 which decreased also decreased when tested with Na. The six cells which were not affected by angiotensin were also not affected by Na. In the ventromedial hypothalamic nucleus (VMH) 10 cells were tested with angiotensin, 3 increased, none decreased, and 7 were not affected. Of the 3 which increased, one also increased in response to Na. Similar results were obtained in the dorsomedial hypo-

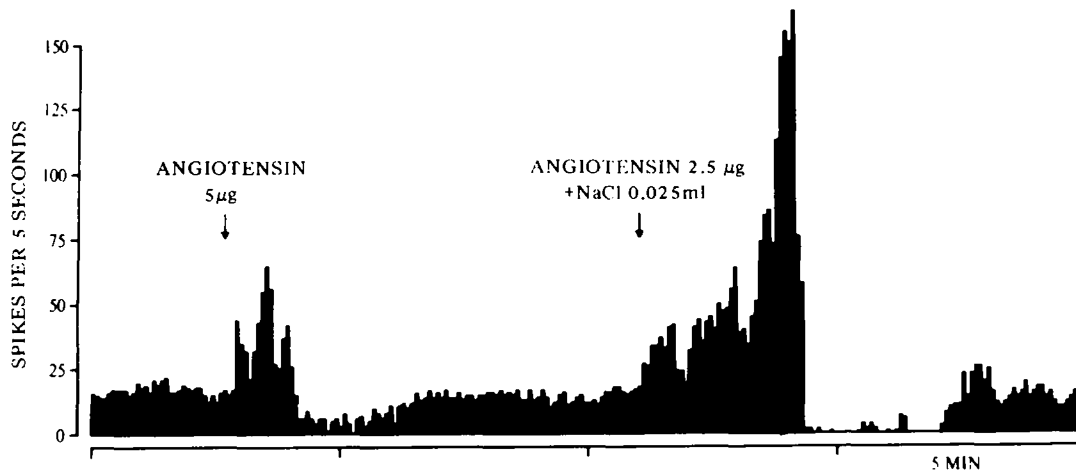

FIG. 4. Similar to Fig. 3 except a different LH-MFB neuron.

TABLE 2

A SUMMARY OF THE EFFECTS OF ANGIOTENSIN II AND Na EJECTED ELECTROPHORETICALLY ON THE NEURONS OF FIVE DIFFERENT BRAIN SITES

Site	ANGIOTENSIN II N	I	D	NE	Na N	I	D	NE	ABL N	I	D	NE
LH	17	0	9	8	2/9	0	1	1	7/9	7	0	0
									1/1 (D)	1	0	0
LH-MFB	34	21	0	13	14/21	3	0	11	3/3	0	3	0
									4/11	0	3	1
					11/13	0	0	11				
Zona Incerta	38	24	2	12	19/24	4	0	15	4/4	0	3	1
									1/12	0	0	1
					2/2	0	1	1				
					9/12	0	0	9				
Thalamus	102	28	7	67	22/28	0	0	22	11/18	2	1	8
					8/67	0	0	8	1/67	1	0	0
					7/7	0	0	7				
Cortex	23	0	1	22	6/22	0	0	6				

thalamic nucleus (DMH). In 6 thalamic cells studied, 2 increased, none decreased and 4 were not affected by angiotensin. Three of the 4 cells which were not affected by angiotensin were tested with Na and 2 decreased and one was not affected. In the dentate gyrus 9 cells were tested with angiotensin, 4 increased, 2 decreased, and 3 were not affected. One of the 4 which increased was tested with Na and it decreased. One of the 2 which decreased was tested with Na and it decreased. In general, cells of the LH-MFB appear to respond to both angiotensin and Na by increasing discharge frequency; whereas, the LH, zona incerta, and dentate gyrus are populated by cells of approximately equal numbers which increase, decrease, or appear to be not affected. Out of a total of 36 cells tested in these three sites, 13 increased, 11 decreased and 12 were not affected by the angiotensin. Cells of the LH-MFB, VMH, DMH and thalamus either increased or were not affected. Of the 43 cells tested in these four sites, 18 increased, none decreased, and 25 were not affected by angiotensin. All of the cells of the LH, LH-MFB and zona incerta which were responsive to

angiotensin responded in the same way to Na.

The latency, duration, and after effects are relatively unreliable and also complicated by tachyphylaxis. These effects will be discussed in greater detail in the next section.

Effects of Electrophoretic Application of Angiotensin II and Na

Data were obtained on 214 neurons in 32 female hooded rats, 245–290 g. The results are summarized in Table 2. Cells of five different brain sites were studied and the number of cells, N, for each site is included in the table. The existence of two different types of LH neuron was confirmed. Of the 17 LH cells studied, 9 decreased (D), none increased (I), and 8 were not affected (NE) by the ejected angiotensin. Of the 9 which decreased, 2 were tested with Na and in one the discharge frequency decreased (D) and the other was not affected. These cells of the lower LH have a relatively low spontaneous discharge frequency which make them difficult to locate; a range of 0–22 spikes per sec with a mean discharge frequency of 4.8 spikes/sec and a standard error of 0.94 spikes/sec. Searching was facilitated by the electrophoretic application of glutamate through one of the capillaries. The effects of glutamate and angiotensin on one of these neurons are illustrated in Figs. 5 and 6. This cell had a spontaneous discharge frequency of about one every 3 sec, as indicated in Fig. 5, which was definitely increased by the ejection of glutamate which continued from the vertical arrow until the end of the fourth tracing. The inhibitory effects of angiotensin ejected by currents of 2, 5 and 20 nA are also illustrated. The duration of the angiotensin application is indicated by the horizontal line. The experiment was repeated on the same cell for ejection currents of 2, 5, 15 and 40 nA, indicated by the horizontal lines, and an X-Y plot of similar data on subsequent tests on the same cell is presented in Fig. 6. Since three barrel electrodes were used and one capillary was filled with glutamate, it was impossible to test any one cell with both angiotensin and Na. The two cells tested for Na therefore did not have enhanced discharge frequencies due to glutamate. Seven of the 9 cells which decreased in discharge frequency increased during electrical stimulation of the basolateral amygdala nucleus (ABL) as indicated in Table 2 and illustrated in Fig. 7. The LH neuron in Fig. 7 decreased during angiotensin application by an ejection current of 100 nA and later increased in discharge frequency during ABL electrical stimulation. The one cell which decreased during both angiotensin and Na ejection also increased when the ABL was stimulated.

Thirty-four LH-MFB neurons were tested. Twenty-one increased, none decreased, and 13 were not affected by angiotensin. The effects of 10, 20 and 100 nA current applied through the angiotensin filled capillary on one cell are illustrated in Figs. 8 and 9. A dose related increase is obvious. A dose related aftereffect is also apparent. As many of these cells had a relatively high spontaneous discharge rate as compared to the lower LH, mean of 9.0 spikes per sec with a standard error of 1.4 spikes per sec, glutamate facilitation was not necessary and 14 out of 21 cells which increased were tested with Na. The effects of angiotensin on an LH-MFB neuron with a relatively high spontaneous dishcarge rate is illustrated in Fig. 10. Three of these cells were increased by Na and 11 were not affected. Of the 13 not affected by angiotensin, the 11 which were tested were not affected by Na. Three of these cells which increased following application of angiotensin and then Na decreased in discharge frequency during basolateral amygdala stimulation. The increase in discharge frequency in an LH-MFB neuron due to angiotensin administered by an ejection current of 100 nA and a later decrease due to basolateral amygdala electrical stimulation is illustrated in

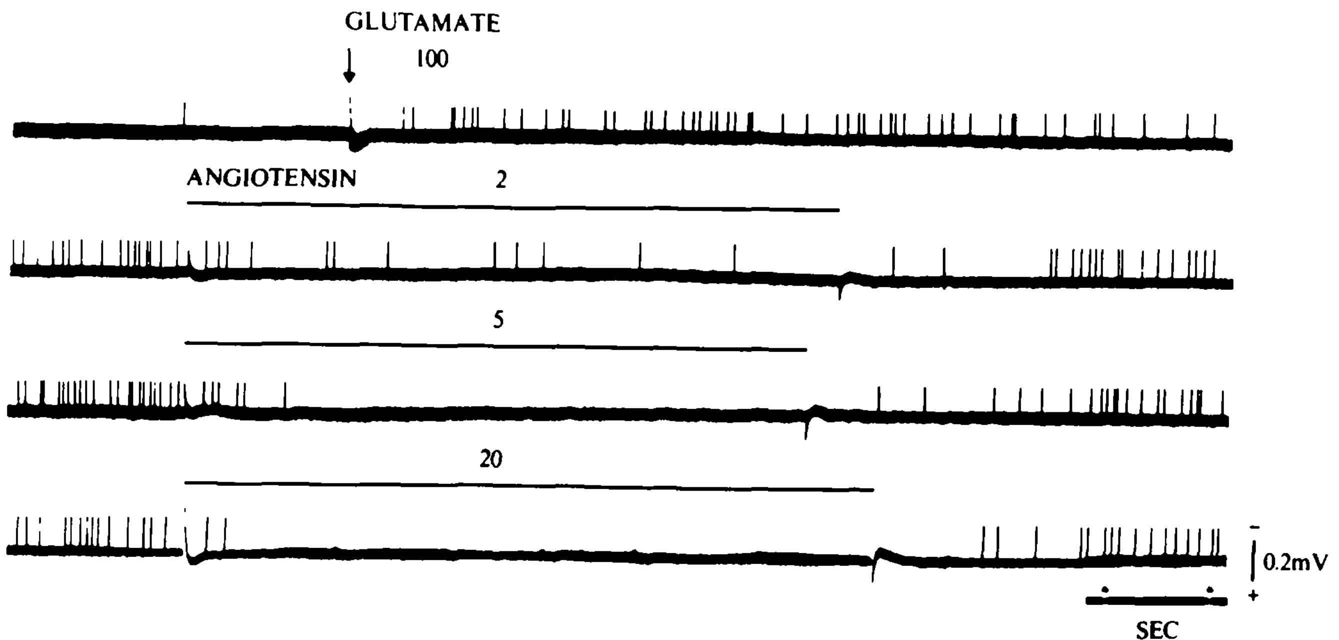

FIG. 5. A neuron of the lower LH. Spontaneous discharges increased by the electrophoretic application of glutamate, from the downward pointing arrow until the end of the fourth tracing, and decreased by ejection of 2, 5, and 20 nA of angiotensin as indicated by the horizontal solid lines.

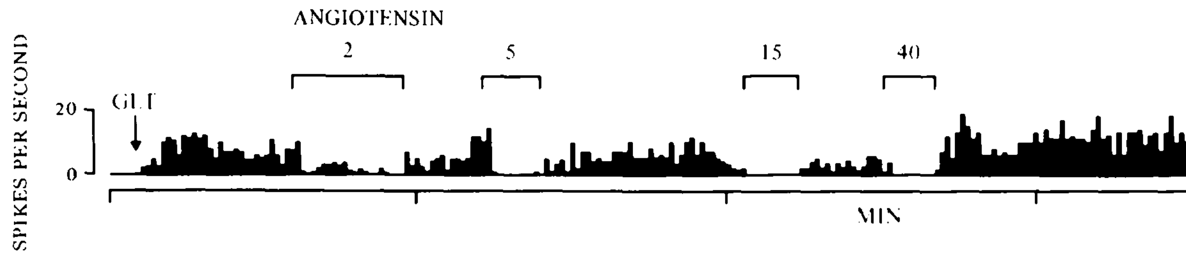

FIG. 6. An X-Y plot of additional data collected on the same cell as in Fig. 5. The downward arrow indicates when the glutamate ejection began and the application of 2, 5, 15, and 40 nA for the ejection of angiotensin indicated by the horizontal lines.

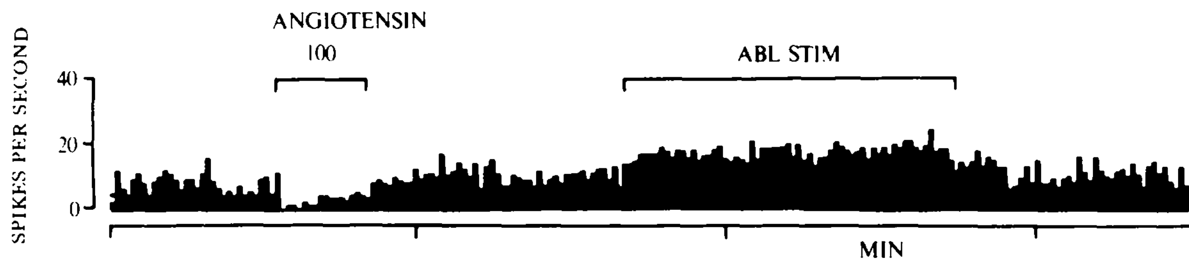

FIG. 7. An X-Y plot of a lower LH neuron illustrating a decrease in discharge frequency due to the electrophoretic ejection of angiotensin by 100 nA and an increase in frequency by electrical stimulation of the basolateral amygdala nucleus (ABL).

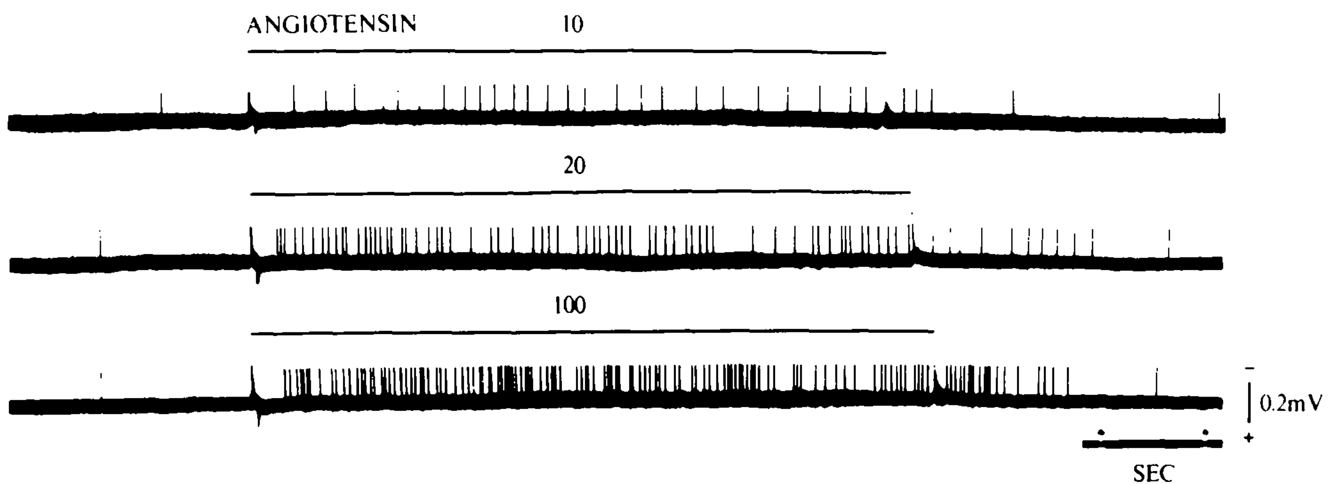

FIG. 8. A neuron of the LH-MFB region which increased in discharge rate during the ejection of angiotensin by 10, 20, and 100 nA.

Fig. 11. Four of the 11 cells which were not affected by either treatment were tested with ABL stimulation, 3 decreased in discharge frequency and the other was not affected.

In the zona incerta 38 neurons were tested. Of these 24 increased, 2 decreased and 12 were not affected. The mean spontaneous discharge rate was 11.7 spikes per sec with a standard error of 2.1 spikes per sec. Of the 24 which were excited by angiotensin, 4 increased in frequency due to Na, and 15 were not affected. Of the 2 which decreased during angiotensin application, one also decreased due to Na. Nine of the 12 cells not affected by angiotensin were also tested by Na and were not affected. The neurons of the LH, LH-MFB, and zona incerta have a low threshold to angiotensin with a required ejection current usually less than 20 nA. The Na sensitive effects had a higher threshold in general and required ejection currents of approximately 100 nA. Three of the 4 zona incerta neurons which increased in response to angiotensin and Na were inhibited by ABL stimulation. In this respect, zona incerta neurons appear to be more similar to the cells of the LH-MFB than those of the more ventral and medial LH.

A relatively large number of the thalamic cells were tested because all of the neurons studied were located along essentially the same electrode tract which resulted from an attempt to place each electrode tip in the same predetermined site. Of the 102 cells tested, 28 increased, 7 decreased in discharge frequency, and 67 were not affected. All of these neurons displayed high thresholds and required more than 50 nA of ejection current to produce an obser-

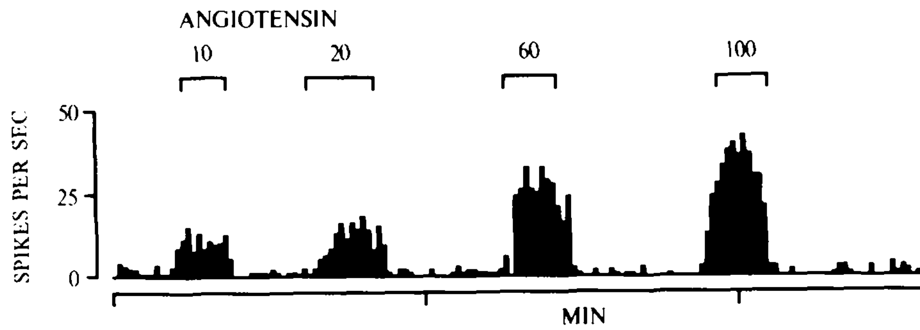

FIG. 9. An X-Y plot of additional data collected on the same neuron in Fig. 8 illustrating a dose related increase in discharge frequency.

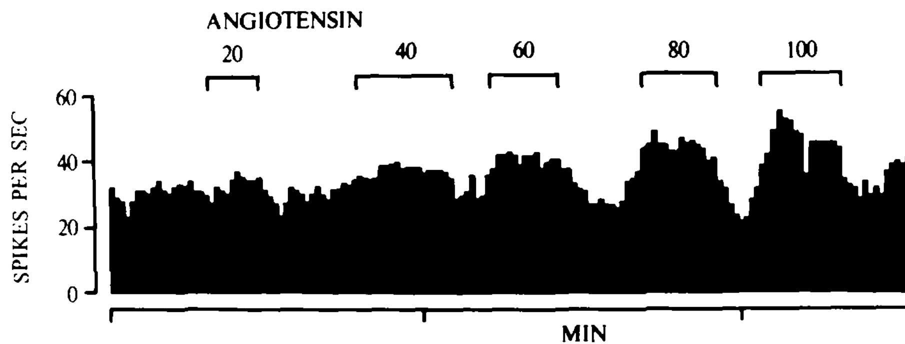

FIG. 10. Same as Fig. 9 except for different LH-MFB cell, one with a higher spontaneous discharge rate.

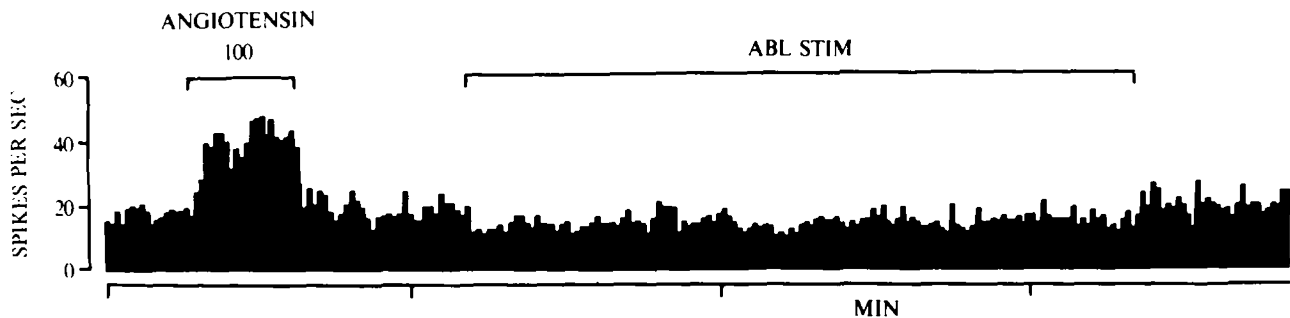

FIG. 11. An X-Y plot of an LH-MFB neuron illustrating an increase in discharge frequency due to the electrophoretic ejection of angiotensin by 100nA and a decrease in frequency by electrical stimulation of the basolateral amygdala nucleus (ABL).

vable increase in frequency. The effects were never as pronounced as in the hypothalamus as illustrated in Fig. 12 where angiotensin was administered by an ejection current of 100 nA. Also the simultaneous application of Na by an ejection current of 100 nA produced very little additional enhancement in discharge frequency. Twenty-two of the 28 thalamic neurons which were increased by angiotensin were tested with Na and were not affected. The 7 which decreased were also tested with Na and were not affected.

In the cerebral cortex 23 cells were tested with angiotensin, one decreased in discharge frequency and 22 were not affected. The decrease in discharge frequency by the one cell required an ejection current of over 200 nA. Six cells were tested with Na and were not affected.

A Comparison of the Effects of Angiotensin II Administered Intravenously and Electrophorectically on the Same Neurons

Because of the possibility that the change in discharge frequency during the electrophoretic application of angiotensin might be due to some inexplicable effect of the ejection current, both intravenous and electrophoretic administration of angiotensin were studied in the same cells. The effects on a typical LH-MFB neuron are illustrated in Fig. 13 where the usual intravenous dose of 5 μg of angiotensin was followed by the electrophorefic application of angiotensin with ejection currents of 100 nA. Similar results are presented for a typical LH low spon-

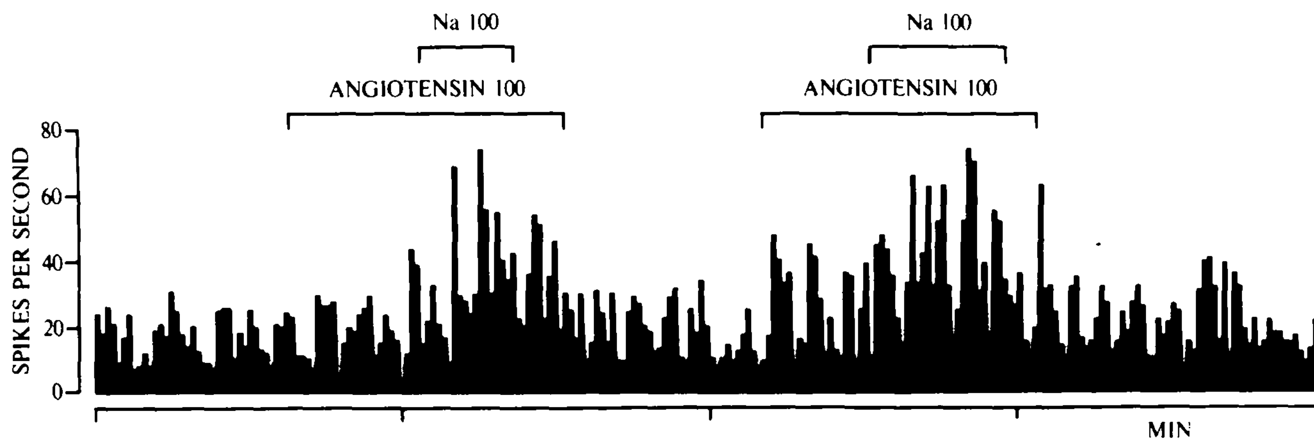

FIG. 12. An X-Y plot of the discharge frequency of a thalamic neuron during electrophoretic angiotensin ejection and combined angiotensin and Na application.

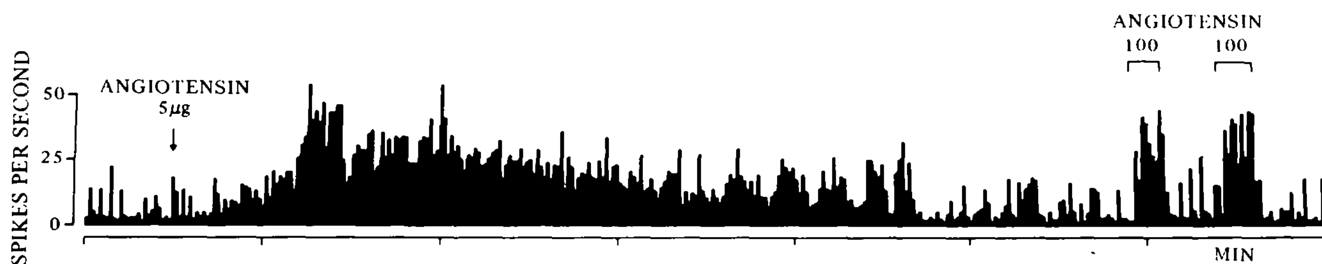

FIG. 13. An X-Y plot of an LH-MFB neuron illustrating an increase in discharge frequency due to an intravenous injection of 5 μg of angiotensin and increases due to two applications of angiotensin by electrophoretic ejection currents of 100 nA.

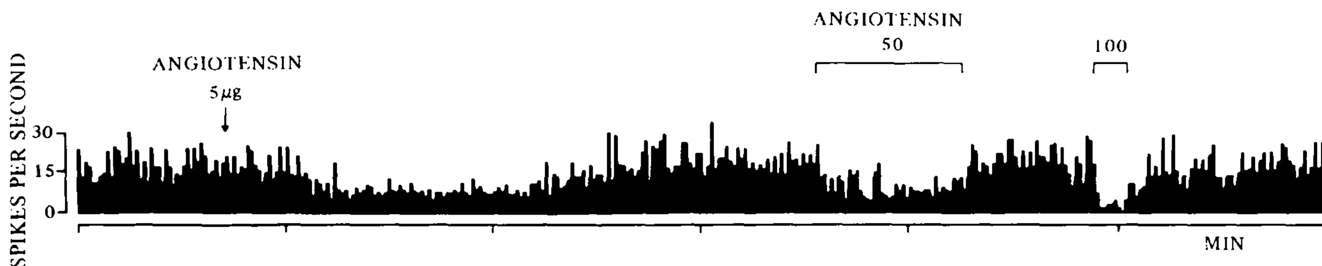

FIG. 14. An X-Y plot of a lower LH neuron illustrating a decrease in spontaneous discharge frequency (enhanced by the continuous electrophoretic application of glutamate) due to the intravenous administration of 5 μg of angiotensin and decreases during the ejection of angiotensin by electrophoretic currents of 50 and 100 nA.

taneously active neuron, in which the discharge rate is being enhanced by the continuous electrophoretic application of glutamate, in Fig. 14. Although it is difficult to measure latencies in low frequency spontaneously active cells when the experimental treatment involves intravenous administration, the time required to reach a peak or maximum effect is definitely much longer for the intravenous administration of angiotensin. The maximum effect is attained almost immediately when applied electrophoretically. The mean latency for cells in the LH, LH-MFB, and zona incerta is 1.7 sec with a range of 0.4–7 sec. The after effect has an average duration of 1.8 sec. As the same dose was administered intravenously in all animals, there is considerable inherent variation in both the latency and duration of the effect as can be seen by an examination of Figs. 13 and 14. In general there seems to be a positive correlation between the data obtained with intravenous and electrophoretic administration of angiotensin. Results on 9 cells tested with both methods is summarized in Table 3. Although the electrophoretic ejection current varied from cell to cell, each cell was tested with an intravenous injection of 5 μg of angiotensin. Cells which were not responsive to the electrophoretic application of angiotensin were not tested with intravenous administration of angiotensin. Of the 6 cells

TABLE 3

A COMPARISON OF ELECTROPHORETICALLY AND INTRAVENOUSLY ADMINISTERED ANGIOTENSIN ON THE SAME NEURONS

	ELECTROPHORESIS			INTRAVENOUS		
Cell	I	D	NE	I	D	NE
1	x			x		
2	x			x		
3	x					x
4	x					x
5	x					x
6	x				x	
7		x			x	
8		x			x	
9		x			x	

which increased during electrophoretic application of angiotensin, 2 increased, 3 were not affected and one decreased when tested intravenously. The 3 cells which decreased during electrophoretic application of angiotensin also decreased when angiotensin was administered intravenously. Although these data are somewhat tenuous, they do support the notion that the electrophoretic effects are due to the angiotensin. As the intravenous administration of angiotensin is confounded by possible secondary effects and the blood brain barrier, the effects of electrophoretic ejection might be easier to interpret.

Potentiation of Angiotensin II Effects by NaCl

In several cells sensitive to both angiotensin and Na, the effect of angiotensin administered intravenously was increased tremendously by the addition of NaCl as illustrated in Figs. 3 and 4. These observations were made only on cells of the LH-MFB and zona incerta. If we combine the data on the LH-MFB and zona incerta, as summarized in Table 4, then of the 72 cells tested with angiotensin, 45 increased, 2 decreased, and 25 were not affected. Thirty-three of these 45 were tested with Na and 7 increased, the other 26 were not affected. Of the 25 cells not affected by angiotensin, 20 were tested with Na and they were not affected. Of the 7 which increased when tested with either angiotensin alone or Na alone, 4 displayed a multiplicative increase or potentiation when tested with angiotensin and Na simultaneously. Of the 20 which were not affected by either the angiotensin alone or Na alone, one displayed the potentiation when angiotensin and Na were administered at the same time.

The potentiation of the effect of angiotensin by Na in an LH-MFB neuron is illustrated in Fig. 15 where the ejection of angiotensin is indicated by a solid horizontal line and the ejection of Na by a broken horizontal line. The experiment was repeated twice with two different doses of angiotensin, ejected by 100 and 120 nA. The increase in discharge frequency due to angiotensin is dose dependent. The administration of 100 nA of Na alone produced a slight but noticable increase in the discharge frequency. However, when applied during the angiotensin ejection, the increase is very pronounced with an obvious post excitatory depression and decrease in action potential amplitude. These changes in the same neuron are presented graphically in Fig. 16 where the successive ejections of angiotensin and Na are indicated by the solid horizontal lines. It is unlikely that the potentiated increase in discharge frequency can be attributed to an additive effect of the ejection currents. First of all the effects are not additive but multiplicative. This is also illustrated in Fig. 17 in another LH-MFB neuron tested first with angiotensin ejected by 200 nA and then with 60 nA of angiotensin plus 100 nA of Na. The 60 nA plus 100 nA produced a greater increase in discharge frequency than 200 nA of angiotensin alone. Second, similar effects can be demonstrated by means of intravenous administration of angiotensin and NaCl as indicated in Figs. 3 and 4.

A typical electrode tract through the cerebral cortex, dentate gyrus, thalamus and LH-MFB is illustrated by the photograph of the unstained formalin fixed 40 μ section in Fig. 18.

DISCUSSION

These results demonstrate clearly that angiotensin II has a direct effect on central neurons. Neurons of the LH, LH-MFB, and zona incerta are relatively more sensitive to angiotensin and Na than cells of the thalamus and cerebral cortex. Many more cells are sensitive to angiotensin than Na and all Na sensitive cells [18] are also sensitive to angiotensin. The low spontaneously active ventral LH neurons decrease in discharge frequency when angiotensin is applied; whereas, the cells of the LH-MFB increase in

TABLE 4

A SUMMARY OF THE SINGLE AND COMBINED EFFECTS OF ANGIOTENSIN II AND Na ON LH-MFB AND ZONA INCERTA NEURONS

ANGIOTENSIN II				Na				COMBINED		
N	I	D	NE	N	I	D	NE	N	I	NE
72	45	2	25	33/45	7	-	26	33/33	4	29
				20/25	-	–	20	20/20	1	19

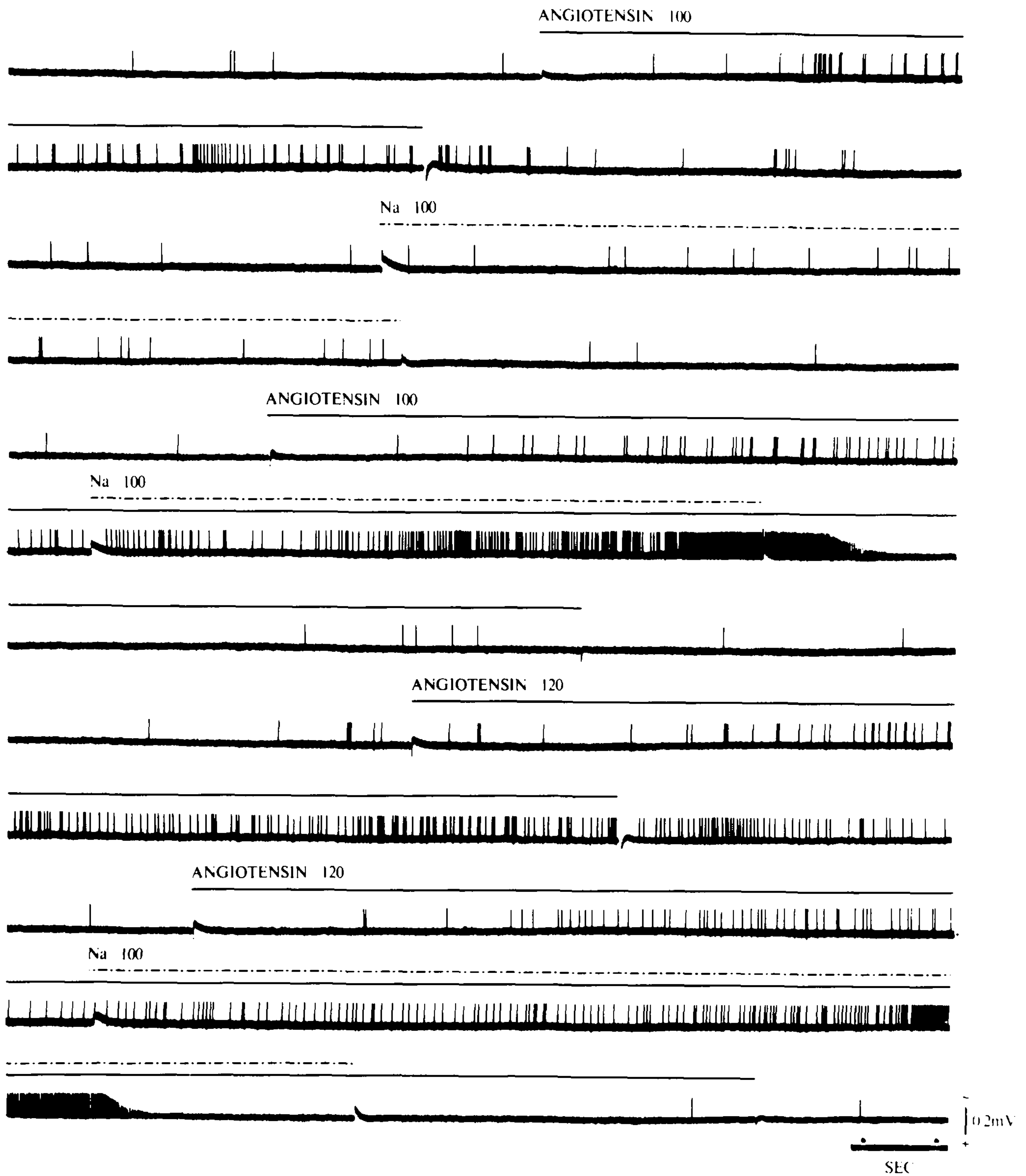

FIG. 15. Continuous unit recording in a LH-MFB neuron. Electrophoretic ejection of angiotensin indicated by the solid horizontal line and the ejection of NA by the broken line. Simultaneous ejection indicated by both solid and broken lines. The experiment was repeated with angiotensin ejection currents of 100 and 120 nA. Voltage and time calibrations as indicated.

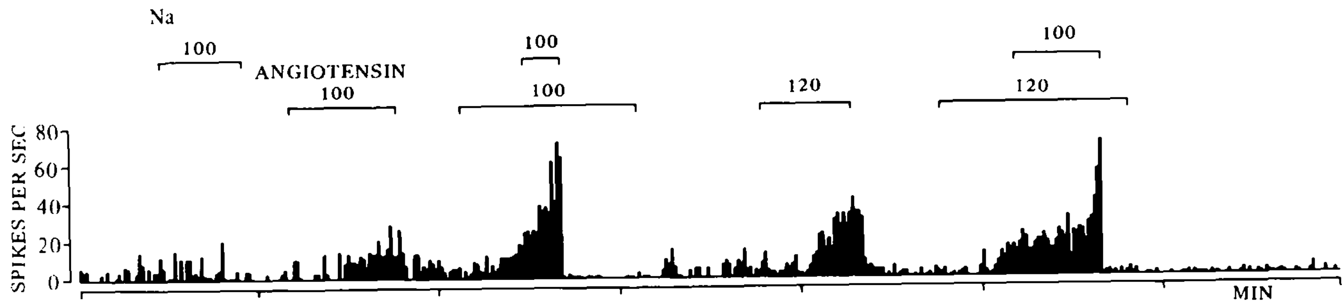

FIG. 16. An X-Y plot of the data for the LH-MFB neuron of Fig. 15.

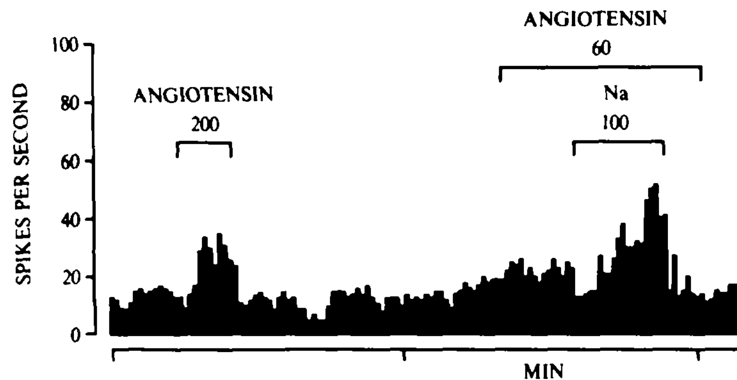

FIG. 17. An X-Y plot of the discharge frequency of an LH-MFB neuron illustrating the multiplicative interaction of angiotensin and Na applied electrophoretically by ejection currents of 160 nA and 200 nA. The combined treatment with a current of 160 nA has a greater effect than angiotensin alone applied by means of 200 nA.

discharge rate. The neurons of the zona incerta are very similar in this respect to the LH-MFB region and possibly represent cells of the same population. In general these results are supported by both the intravenous and electrophoretic ejection data. Because of the relatively crude histological methods which were utilized, it is possible that the three LH neurons which increased with angiotensin, in Table 1, were actually in the LH-MFB and, for the same reasons, the two zona incerta neurons which decreased during angiotensin administration, in Table 2, were actually in the LH. The existence of two distinct types of lateral hypothalamic neurons is further substantiated by the effects of electrical stimulation of the basolateral amygdala nucleus. The lower LH neurons which decrease in discharge frequency in response to angiotensin are excited by ABL stimulation. The data on ABL stimulation indicates that cells of the LH-MFB and zona incerta which respond to both angiotensin and Na are inhibited by ABL stimulation. Identification of spontaneously active neurons by afferent or antidromic stimulation seems essential for future studies. Even with these limitations, angiotensin seems to have a specific effect on two types of neurons in the ventral LH and LH-MFB-zona incerta regions. Many cells of the LH-MFB-zona incerta increased in frequency at relatively low doses of angiotensin applied electrophoretically which might be attributed to an increase in sodium permeability as these cells are also sensitive to Na. In this respect, these cells respond more like typical receptors and also display dose related effects. The cells of the LH which decrease in discharge frequency might be similar to smooth muscle where the outward sodium pump is enhanced by angiotensin [22] and a hyperpolarization of the membrane would explain the decrease in frequency.

With larger doses the effects of angiotensin appear to be more nonspecific and many cells of the thalamus are also affected. Although a few of these cells were affected by basolateral amygdala stimulation, none responded to Na. None of the cerebral cortex cells responded to Na. With intravenous administration of NaCl several cells in both the thalamus and dentate gyrus were affected and the changes

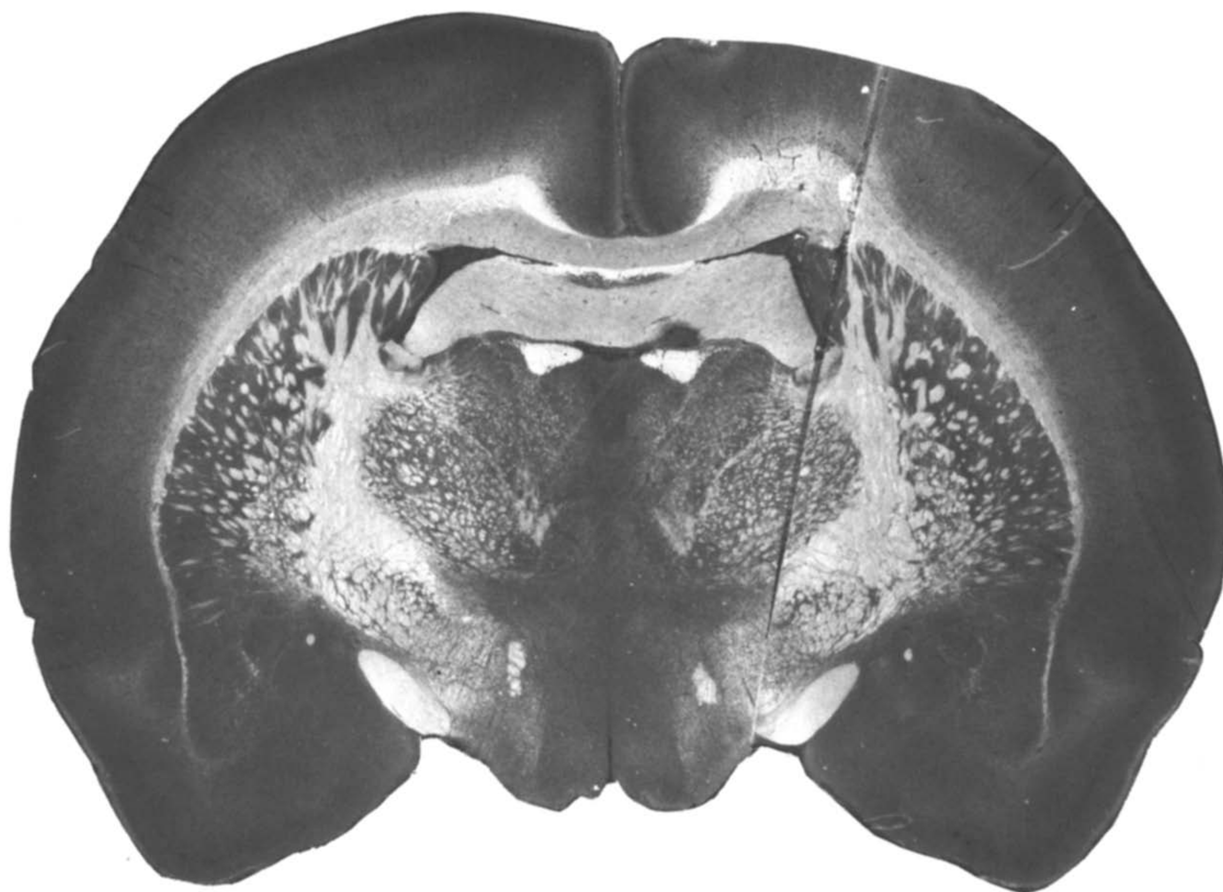

FIG. 18. A photograph of an unstained 40 μ formalin fixed section of a rat brain illustrating a typical electrode tract into the LH-MFB.

can possibly be attributed to some secondary neural effect. The possibility of secondary effects resulting from capillary contraction caused by angiotensin seems unlikely since the angiotensin sensitive neurons are also facilitated by acetylcholine and isoproterenol [19]. The possibility that the so-called angiotensin sensitive hypothalamic neuron is actually responding to changes in arterial blood pressure [10] produced by the intravenous administration of angiotensin is difficult to explain without additional data. However, the results of the electrophoretic experiments do not support such an explanation. In the experiments which utilized intravenous administration of angiotensin the effect had the following mean characteristics: latency 35.4 ± 5.4 sec (standard error), latency to peak effect 56.8 ± 6.5 sec, and duration 181.3 ± 18.0 sec. When carotid artery blood pressure was measured in rats under similar conditions of urethan anesthesia, 5 μg of angiotensin II produced the following mean change in blood pressure: latency 9.0 ± 1.1 sec, latency to peak effect 30.4 ± 2.4 sec, and duration 218.6 ± 8.9 sec. Even though the time courses are reasonably different, independent manipulation of blood pressure when recording from hypothalamic angiotensin sensitive neurons should be carried out in the future.

These results suggest that angiotensin facilitates membrane Na permeability. The data confirm similar effects on drinking and blood pressure induced by intraventricular administration of the same substances [2,3]. Drinking induced by intracerebral application of angiotensin is enhanced at threshold doses if the administration vehicle contains Na and decreased if it is mixed with K [21]. It is also interesting that ethyl alcohol, angiotensin, and Na combine to produce a multiplicative interaction which results in a pronounced increase in lateral hypothalamic neuronal activity [27]. Both angiotensin [22] and ethyl alcohol [12] affect sodium transport mechanisms in excitable membrane. As Na complexes with valine^5-angiotensin II to form a more biologically active molecule by changing the steric conformation of the peptide chain [4], it is not possible under present conditions to determine if the interaction is due to independent effects of both treatments on the membrane.

These results tend to support the hypothesis that ". . . the activity of brain receptors involved in the control of water balance is influenced by variations in the environmental Na^+ ion concentration rather than by osmotically induced changes in the receptor volume." [1, p. 209] Angiotensin and ethyl alcohol in small quantities might influence drinking because of specific effects on ion transport mechanisms in Na sensitive hypothalamic neurons. The fact that eating can be produced by altering hypothalamic ionic concentrations has also been recently established [14,15].

REFERENCES

1. Andersson, B. Receptors subserving hunger and thirst. In: *Handbook of Sensory Physiology*, edited by E. Neil, Vol. 3, Enteroceptors. New York: Springer-Verlag, 1972. pp. 187–216.
2. Andersson, B. and O. Westbye. Synergistic action of sodium and angiotensin on brain mechanisms controlling fluid balance. *Life Sci.*, Part 1,**9**: 601–608, 1970.
3. Andersson, B., L. Eriksson and O. Fernández. Reinforcement by Na+ of centrally mediated hypertensive response to angiotensin II. *Life Sci.*, Part 1, **10**: 633–638, 1971.
4. Bergmann, P., J. Oehme, J. Jelínek and J. H. Cort. Potentiation of angiotensin and eledoisin activities by sodium chloride. *Life Sci.*, Part 1, **10**:969–975, 1971.
5. Curtiss, D. R. and K. Koizumi. Chemical transmitter substances in brain stem of cat. *J. Neurophysiol.* **24**: 80–90, 1961.
6. Daniels-Severs, A., E. Ogden and J. Vernikos-Danellis. Centrally mediated effects of angiotensin II in the unanesthetized rat. *Physiol. Behav.* **7**: 785–787, 1971.
7. Epstein, A. N., J. T. Fitzsimons and B. J. Rolls. Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol. (Lond.)* **210**: 457–474, 1970.
8. Fitzsimons, J. T. The renin-angiotensin system in the control of drinking. In: *The Hypothalamus*, edited by L. Martini, M. Motta and F. Fraschini. New York: Academic Press, 1970. pp. 195–212.
9. Fitzsimons, J. T. and B. J. Rolls. The effect of angiotensin on drinking in the rat. *J. Physiol. (Lond.)* **196**: 39–41, 1968.
10. Frazier, D. T., C. Taquini, L. L. Boyarsky and M. F. Wilson. Hypothalamic unit resonses to increases in arterial blood pressure. *Proc. Soc. exp. Biol. Med.* **120**: 450–454, 1965.
11. de Groot, J. *The Rat Forebrain in Stereotaxic Coordinates.* Verhandelinger der koniunklijke Nederlandse Akademie van Wetenschappen, Afd. Natuurkunde. (N. V. Noord-Hollandsche Uitgevers Maatschappej, Amsterdam.) 1959.
12. Israel, Y., E. Rosenmann, S. Hein, G. Colombo and M. Canessa-Fischer. Effects of alcohol on the nerve cell. In: *Biological Basis of Alcoholism*, edited by Y. Israel and J. Mardones. New York: Wiley-Interscience, 1971. pp. 53–72.
13. Johnson, A. K. Localization of angiotensin sensitive areas for thirst within the rat brain. Paper read at EPA Meetings in Boston, April, 1972.
14. Myers, R. D. and W. L. Veale. Spontaneous feeding in the satiated cat evoked by sodium or calcium ions perfused within the hypothalamus. *Physiol. Behav.* **6**: 507–512, 1971.
15. Myers, R. D., S. A. Bender, M. K. Krstic and P. D. Brophy. Feeding produced in the satiated rat by elevating the concentration of calcium in the brain. *Science* **176**: 1124–1125, 1972.
16. Nicoll, R. A. and J. L. Barker. Excitation of supraoptic neurosecretory cells by angiotensin II. *Nature* **233**: 172–173, 1971.
17. Osborne, M. J., N. Pooters, G. Angles d'Auriac, A. N. Epstein, M. Worcel and P. Meyer. Metabolism of tritiated angiotensin II in anesthetized rats. *Pflügers Arch. ges. Physiol.* **326**: 101–114, 1971.
18. Oomura, Y., T. Ono, H. Ooyama and M. J. Wayner. Glucose and osmosensitive neurons of the rat hypothalamus. *Nature* **222**: 282–284, 1969.
19. Oomura, Y., M. Sugimori, T. Nakamura, D. Gawronski and R. Fukuda. Catecholamine effects on angiotensin receptive neurons in the rat hypothalamus. Abstract IUPS Regional Meeting, Sydney, Australia, 1972.
20. Routtenberg, A. Intracranial chemical injection and behavior: a critical review. *Behav. Biol.* **7**: 601–641, 1972.
21. Swanson, L. W., L. G. Sharpe and D. Griffin. Drinking to intracerebral angiotensin II and carbachol: dose-response relationships and ionic involvement. *Physiol. Behav.* **10**: 595–600, 1973.
22. Turker, R. K., I. H. Page and P. A. Khairallah. Angiotensin alteration of sodium fluxes in smooth muscle. *Arch. int. Pharmacodyn.* **165**: 394–404, 1967.
23. Volicer, L. and C. G. Loew. Penetration of angiotensin II into the brain. *Neuropharmacology* **10**: 631–636, 1971.
24. Wayner, M. J., T. Ono and D. Nolley. Effects of angiotensin applied electrophoretically on lateral hypothalamic neurons. *Pharmac. Biochem. Behav.* **1**: 223–226, 1973.
25. Wayner, M. J., R. Peterson and A. Floczyk. A constant current device for brain stimulation. *Physiol. Behav.* **8**: 1189–1191, 1972.
26. Wayner, M. J., D. Gawronski, C. Roubie and I. Greenberg. Effects of ethyl alcohol on lateral hypothalamic neurons. In: *Recent Advances in Studies of Alcoholism*, edited by N. Mello and J. Mendelson. Washington, D. C.: U.S. Government Printing Office, 1971. pp. 219–273.
27. Wayner, M. J., T. Ono, D. Nolley and A. De Young. Effects of ethyl alcohol, angiotensin, and several amino acids on the lateral hypothalamus. In: *Recent Studies of Hypothalamic Function*, edited by K. Cooper, K. Lederis and W. Veale. Basel/New York: S. Karger, 1973. (in press)