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Arginine^8-Vasopressin Enhances the Responses of Lateral Septal Neurons in the Rat to Excitatory Amino Acids and Fimbria-Fornix Stimuli*

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In the present study we investigated the effect of arginine^8-vasopressin (AVP) on responses induced in lateral septal neurons of the rat by iontophoretically administered excitatory and inhibitory amino acids and by synaptical stimuli delivered through fimbria-fornix (fi-fx) fibers. In the majority of the lateral septal neurons, iontophoretically applied AVP induced a marked increase in the excitatory responses to glutamate, aspartate, quisqualate and N-methyl-D-aspartate. The responses to excitatory amino acids frequently remained elevated several minutes after termination of the peptide administration. Inhibitory responses induced by GABA were not affected by AVP. The responsiveness of lateral septal single units to fi-fx stimuli was enhanced during iontophoretic administration of AVP. The enhanced responsiveness also appeared from experiments in which topically applied AVP induced a prolonged increase in the negative but not the positive wave of field potentials evoked in the lateral septum by fi-fx stimuli. The possible physiological significance of these findings is discussed.

INTRODUCTION

Immunohistochemical studies[1,20] have revealed that the lateral septal complex (LSC) of rats is rich in arginine^8-vasopressin (AVP) containing fibers which originate in hypothalamic neurosecretory neurons[1,8,20,23]. It appeared that many of the vasopressinergic fibers make synaptic contacts with the cells in the LSC[2]. The vasopressinergic terminals in the LSC are also capable of releasing the peptide, since septal tissue depolarized by K^+ or veratridine released radioimmunoassayable amounts of AVP in presence of Ca^{2+}. These findings, the increased frequency of hippocampal theta rhythm[22] and delayed extinction of avoidance behavior[13] observed in rats after microinjection of AVP (1 and 25 pg, respectively) into the septum support the idea that synaptically released AVP may affect the activity of LSC neurons.

Recently, we examined the effect of iontophoretically administered AVP on neurons in the LSC[10]. It appeared that ca. 40% of the LSC neurons were excited by AVP, administered with rather high currents. The remaining neurons were not affected by AVP even when the peptide was expelled with currents of up to 200 nA. In a number of these cells however, at very low expelling currents AVP increased the responses of the cells to glutamate (GLU). The AVP-induced change of neuronal responses to a chemical stimulus seemed of interest since it may be accompanied by changes in the responsiveness to synaptical stimuli from the fimbria-fornix (fi-fx) fibers, which constitute a major afferent[16,21], glutamatergic[11,12,24] projection to LSC neurons.

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In the present study, we first firmly established the effect of iontophoretically administered AVP on GLU-evoked excitations in LSC neurons. To provide some indications on the specificity of the effect we also investigated the action of the peptide on responses to aspartate (ASP), N-methyl-D-aspartate (NMDA) and quisqualate (QUIS), which were reported to excite LSC cells[12], and on responses to an inhibitory agent in the LSC[7,15,19], i.e. GABA. Subsequently, we investigated whether changes in the responses to exogenously administered compounds induced by AVP were accompanied by alterations in the cell responses to transsynaptical stimuli. In LSC single units, responding to fi-fx stimulation with an excitation followed by a period of inhibition[5,6,10,19] we established the responsiveness to fi-fx stimuli before, during and after iontophoretic application of AVP. In another experimental paradigm, we tested the effect of topically applied AVP on the field potential (FP) which is associated with the excitation-inhibition responses of LSC single units to fi-fx stimulation, to get some indication about the effective doses of AVP. Apart from AVP, the effect of topically applied GLU, GABA and muscimol on the FPs is described in order to compare these effects with the peptide induced action.

MATERIALS AND METHODS

The experiments were performed on 69 male Wistar rats (250–300 g) anesthetized with urethane (1.5 g/kg). Following craniotomy, the neocortex and corpus callosum overlying the septum were removed by suction. In this preparation, the intersection of the anterior border of the hippocampal commissure with the midline served as reference zero. Surgical procedures are described in more detail elsewhere[11].

Stimulation and recording

A comb of 4 pairs of 100 μm stainless steel electrodes were unilaterally inserted into the fi-fx immediately behind the caudal border of the hippocampal commissure. These electrodes served for the transsynaptic activation of single units and for the induction of FPs in the LSC.

Action potentials of single neurons were recorded extracellularly with glass micropipettes. The recording electrodes were glued to a 7 barrelled multipipette so that their tips protruded in front of the multipipette by 30–50 μm. Action potentials were amplified, displayed and counted by techniques previously described[11].

Field potentials were recorded through 40 μm stainless steel wire. Unfiltered FPs were amplified and stored on magnetic tape for further analysis.

Drugs

The channels of the multibarrelled pipettes were filled with solutions of the following drugs: L-aspartic acid (ASP, Sigma, 0.25 M adjusted to pH 8 with NaOH, expelled as the anion); L-glutamic acid (GLU, Sigma, 0.25 M, pH 8, anion); γ-aminobutyric acid (GABA, Sigma, 0.1 M, adjusted to pH 4 with HCl, cation); bicuculline (Sigma, 10 mM, pH 4, cation) all aqueous solutions; N-methyl-D-aspartate (NMDA, CRB, 50 mM); quisqualic acid (QUIS, CRB, 5 mM) and arginine[8]-vasopressin (AVP, Organon, 5 or 10 mM) all dissolved in 165 mM NaCl, pH unadjusted and ejected as the anion.

Automatic current balancing was performed through a barrel containing 3 M NaCl. Retaining current for the peptides was 0–5 nA, for the other compounds ca. 10 nA.

Experimental protocol and data analysis

Single unit experiments. Neurons were sampled in penetrations made through the lateral part (600–900 μm from the midline) of the LSC, ca. 1500 μm anterior of the reference zero and 0–1500 μm below the surface. Prior to the microiontophoretic applications of drugs, the response of the neurons to fi-fx stimuli was established. LSC neurons which invariably responded to fi-fx stimuli with an action potential at a stable latency of ca. 5 ms and followed stimulus frequencies of 50–150 Hz were considered to be orthodromically activated.

In these neurons the effect of AVP on the neuronal activity was tested in the following procedure. First, AVP was administered with currents of increasing intensity. If the spontaneous activity of the recorded neuron did not change during application of the peptide, the effect of AVP on responses to iontophoretically administered amino acids was studied. The amino acid was applied at regular intervals (30 s) with fixed current intensity until the response became stable. Subsequently, the responses to the amino acid

were tested while AVP was delivered through two barrels simultaneously with 5 nA. If AVP had no effect or changed the amino acid response by less than 50%, the expelling current of AVP was increased and the effect of AVP on the amino acid response was tested once more. This procedure was repeated as long as the criterion of 50% change in response was not met (maximal current for AVP, 50 nA delivered through two barrels simultaneously). Whenever possible, series of at least 3 amino acid responses, i.e. before, during and after delivery of the peptide at an effective current level were recorded in at least two test-runs.

The effect of AVP on synaptically induced excitations was examined in a test in which 32 stimuli were delivered at threshold stimulus intensity (stimulus-response ratio ca. 2) before, during and after iontophoretic release of the peptide. First, the peptide was expelled with currents which were shown to enhance the responses to excitatory amino acids. If AVP failed to affect the synaptic response at this current level, the expelling current was increased stepwise and the test was carried out once more. This procedure was repeated as long as AVP did not increase the synaptic activity by 50% (maximal current for AVP, 50 nA delivered through two barrels simultaneously). Post-stimulus histograms of the neuronal response before, during and after administration of AVP were computed in a computer average transient set into the post-stimulus histogram mode.

Field potentials. FPs were recorded in the LSC at 1500 μm anterior of the zero, 750 μm lateral from midline and at a depth of 100–200 μm below the surface. Prior to the experiment, the pair of fi-fx electrodes which induced the largest FPs was determined and the stimulus intensity adjusted to elicit a FP of ca. 50% of the maximal amplitude. This pair of electrodes and stimulus intensity were used through the rest of the experiment. As soon as the FPs attained stability (usually within 2 h after surgery) the experiment was started. In 8 control experiments, we established the effect of time and manipulations associated with repeated topical administrations on the FPs. In these experiments, at time t = −45 min 10 μl of artificial cerebrospinal fluid (ACSF, 154 mM NaCl; 2.81 mM KCl; 1.45 mM $CaCl_2 \cdot 2H_2O$; 0.76 mM $MgSO_4 \cdot 7H_2O$; 0.16 mM Inuline (Merck) in distilled water, pH 7.4) was applied on the dorsal surface of the septum and FPs elicited by 32 monopolar fi-fx stimuli were recorded on magnetic tape. At t = −30 min the ACSF was replaced by the same amount of fresh ACSF and new series of FPs were recorded. This procedure was repeated at t = −15, 0, 90 and 120 min. Additional series of FPs which were not preceded by any application were recorded at t = 15, 30 and 60 min (see x-axis in Fig. 3). The experimental rats were subjected to the same procedure as the control rats except that at t = 0 min, the ACSF was replaced by 10 μl of 10^{-8}–10^{-10} M AVP. At t = 90 min, the preparation was washed out at least 3 times with ACSF before new series of FPs were recorded.

The effect of GLU, GABA and of muscimol on the magnitude of the FPs was studied in 12 rats. In these experiments, we recorded FPs induced by 32 stimuli delivered every 5 min to fi-fx fibers while the dorsal surface of the septum was continuously superfused (0.2 ml/min) with warm oxygenated (5% CO_2 + 95% O_2) ACSF or with one of the drugs dissolved in ACSF.

FPs with appropriate calibration signals were fed into a minicomputer which computed, printed and plotted averaged FPs ± S.E.M. (standard error of the mean) for each series of stimuli. In addition, it computed the magnitudes of the negative and positive waves in the averaged FPs. For the AVP experiments, magnitudes of the negative and positive waves in the averaged FPs were expressed as percentages of the corresponding values obtained for the averaged FP at t = −45 min in the same experiment.

Histology

At the end of each experiment, positions of the stimulating and recording electrodes were marked for later histological examination as described elsewhere[11,12].

TABLE I

The effect of iontophoretically applied AVP on responses induced in lateral septal neurons by glutamate (GLU), aspartate (ASP), quisqualate (QUIS) and N-methyl-D-aspartate (NMDA)

	GLU	*ASP*	*QUIS*	*NMDA*
Increase	45	9	9	18
Decrease	2	1	1	2
No effect	14	2	8	6

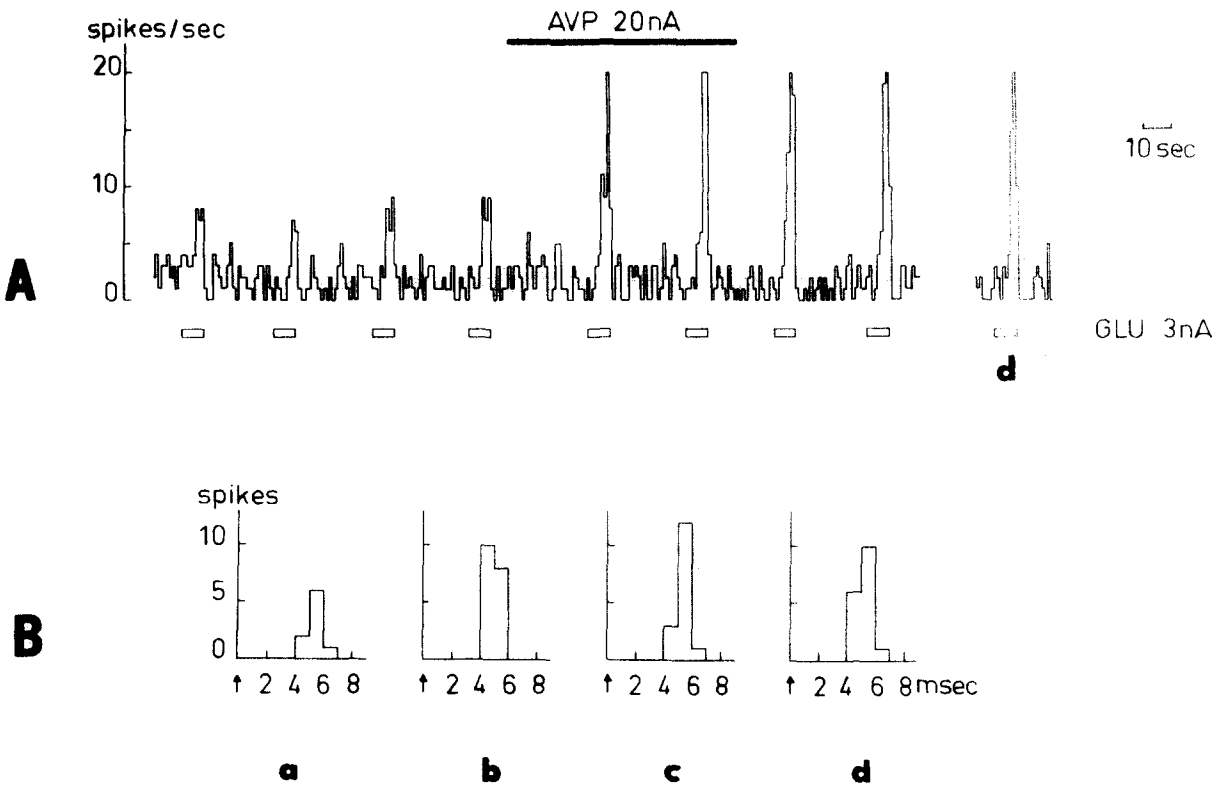

Fig. 1. A: responses to glutamate (GLU) established in a lateral septal neuron before, during and after concomitant iontophoretic release of AVP. The ordinate represents the number of spikes per second. Horizontal bars indicate the duration of the iontophoretic applications. The recording at the right indicated by d was obtained 10 min after termination of the AVP-administration. B: post-stimulus histograms showing the responses of the same neuron to 32 fi-fx stimuli delivered before the AVP delivery (a), during application of AVP (20 nA for 1 min, b) and during the control obtained 5 min (c) and 10 min (d) after termination of the peptide administration. Arrows indicate the moment of stimulation.

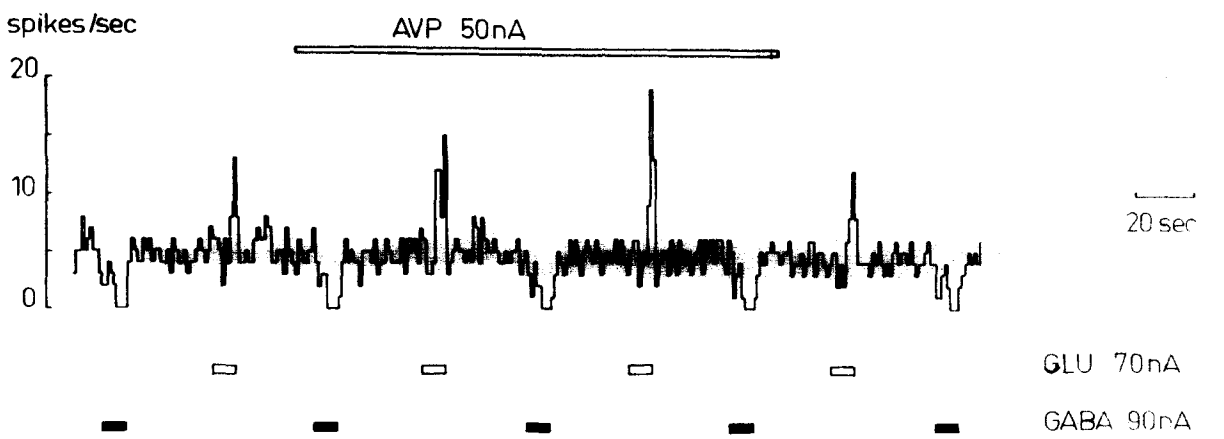

Fig. 2. Responses to alternately applied glutamate (GLU) and GABA recorded in a lateral septal neuron before, during and after administration of AVP. All compounds were applied iontophoretically. The ordinate represents the number of spikes per second. Horizontal bars indicate the duration of the application.

RESULTS

Single unit experiments

Approximately 30% of the LSC neurons tested were excited by AVP iontophoretically applied generally with high currents (100–150 nA). These neurons were excluded from further investigations. In the remaining neurons (n = 89) we studied the effect of AVP on amino acid-induced responses.

In ca. 75% of the neurons tested, AVP increased the responses to GLU by more than 50% (see Table I). In the majority of the neurons the peptide-induced effect was observed in repeated tests. In 7 cells though, AVP enhanced the GLU-evoked responses only once, while on the second application the peptide turned out to be ineffective. Enhancement of the GLU-responses was obtained with AVP-expelling currents ranging from 10 to 100 nA (41.1 ± 4.9 nA, mean ± S.E.M.). In ca. 60% of the cells tested, the response to GLU returned after termination of the AVP administration to the pre-treatment level. In the remaining 40% of the neurons (see an example in Fig. 1) though, the responsiveness to GLU remained elevated for a period that could last up to 15 min (maximal testing interval).

Excitations elicited in LSC neurons by ASP, QUIS and NMDA were affected by AVP in a similar way as the responses to GLU (see Table I). Thus, in the majority of the neurons the responses to either of these amino acids increased markedly during concomitant release of AVP. In ca. 50% of the cells, responses remained increased for 3–15 min after termination of the peptide application. In 11 cells, the effect of AVP on responses to GLU was compared to that on excitations induced by one or more of the other amino acids. It was observed that in 9 of these cells, AVP expelled with currents which increased the responses to GLU also appeared to be effective in enhancing the responses to one or more of the other excitatory amino acids.

GABA, iontophoretically administered with low (5–25 nA) currents, readily depressed the spontaneous activity of all LSC neurons tested (n = 34). In 16 of 21 neurons investigated, the GABA-induced inhibition was reversed by concomitant release of bicuculline, indicating that the inhibition was presumably mediated by some kind of GABA-sensitive receptors on LSC cells. AVP was not able to alter GABA-evoked inhibitory responses in any of 12 neurons thus tested, although in all of these neurons the peptide did enhance the excitatory responses to GLU (see Fig. 2).

The effect of AVP on transsynaptic excitations was studied in 18 orthodromically activated LSC cells. In 12 of these neurons iontophoretic administration of AVP with currents that increased responses to GLU, was associated with an increase of at least 50% in the number of action potentials elicited by 32 fi-fx stimuli. Post-stimulus histograms of the responses obtained at 5, 10 and 15 min after termination of the AVP administration indicated that in 8 cells the number of responses to stimulation returned to the pre-treatment level within 5 min. In 4 neurons though, the number of synaptic responses remained augmented for the rest of the testing interval (see an example in Fig. 1).

In 6 neurons, AVP enhanced the GLU-induced responses but failed to affect the responsiveness to stimulation of the fi-fx.

Field potential experiments

FPs elicited by fi-fx stimuli in the dorsolateral part of the septum exhibited a large negative wave of short duration followed by a smaller and broader positive wave. The mean latency to the peak of the negativity (5.2 ± 0.4 ms, mean ± S.E.M.) did not differ significantly from the mean latency observed for the inducement of action potentials in LSC cells (4.8 ± 0.2 ms) in response to the same stimuli. The marked potentiation of FPs occurring with interstimulus intervals of 20–40 ms (not shown) indicated the synaptic character of the FPs. We may therefore assume that the FPs in response to fi-fx stimuli obtained in the present experiments are the extracellular counterpart of the postsynaptic responses in LSC cells and do not represent a synchronous volley in the fibers crossing the LSC.

As illustrated in Fig. 3 for a group of 8 control rats, the size of the negativity in the FP decreased during the experiment. The mean negative wave in FPs recorded 3 h after the start of the experiment amounted on average to 80% of the negativities evoked by the first series of stimuli. The positive wave of the FPs changed little during the experiment. Topical administration of 10^{-8} M AVP in ACSF markedly altered the time related decrease of the

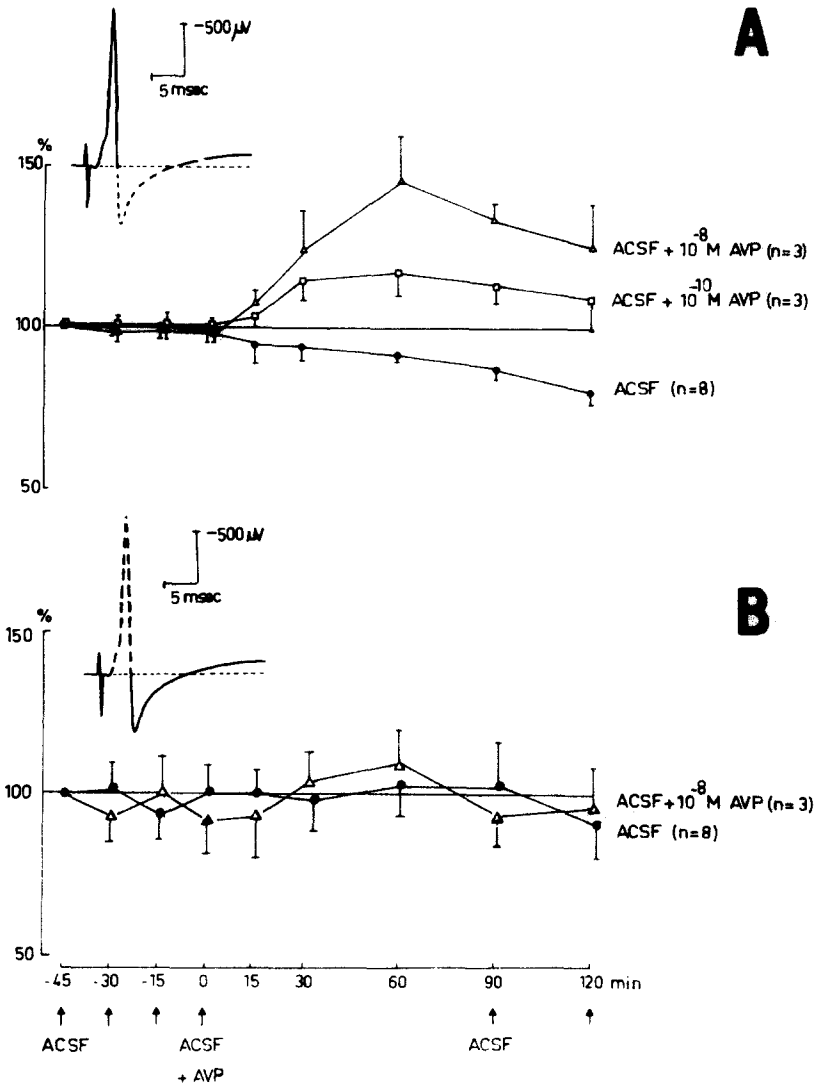

Fig. 3. Mean amplitude ± S.E.M. (standard error of the mean) of the negative (A) and positive (B) waves of averaged field potentials (see an example in insets) obtained in experiments in which either artificial cerebrospinal fluid (ACSF) or 10^{-8} M and 10^{-10} M AVP in ACSF were applied topically on the dorsal surface of the septum. For each experiment the amplitudes were expressed as percentages of the amplitude obtained for the first field potential of the experiment obtained at t = −45 min. Arrows indicate the moment of a single topical application of ACSF or AVP dissolved in ACSF.

negative waves. Already 15 min after the application of AVP, the mean negative waves reached considerably higher values than the mean negativities recorded at the same time for the control rats. The magnitude of the negativity in the peptide treated rats continued to increase whereas in the control rats it steadily declined. Sixty min after the topical application of AVP, the difference in size of the negativity between both groups of rats amounted to ca. 50%. A 3-times repeated washing-out of the peptide with ACSF at t = 90 min did not induce any abrupt decrease in the amplitude of the negative waves. Instead, the negativities recorded after the wash-out declined at a rate similar to that observed in the control group so that the marked difference in magnitude of the negative waves between the two groups of rats remained apparent until the end of the experiment. The positive wave of the FPs did not show any consistent alterations in presence of topically applied AVP.

Fig. 3 illustrates that 10^{-10} M AVP still induced a clear increase in the size of the negative-going FPs. Lower concentrations of the peptide appeared to be

ineffective (not shown).

Superfusion of the septum with 10^{-3} or 5×10^{-3} M GLU in ACSF decreased both the negative and positive waves of the FP in a dose-dependent way (see Fig. 4A). Lower concentrations of GLU appeared to be ineffective. A short duration (5 min) superfusion of the septum with GABA (effective concentrations 10^{-2} or 5×10^{-2} M, see Fig. 4B) and muscimol (10^{-4} M) induced a marked decrease of the positive wave accompanied by a mild increase of the negative wave.

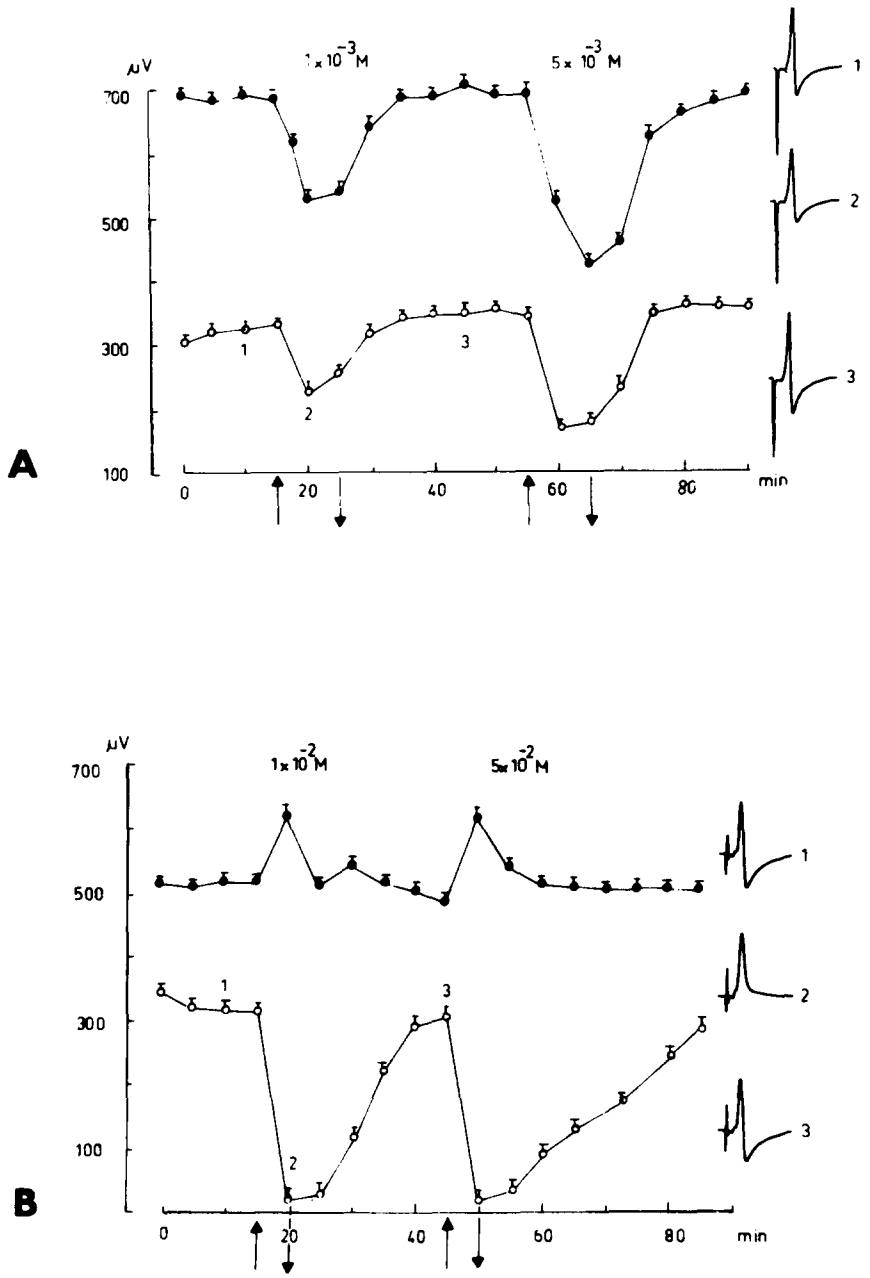

Fig. 4. Mean amplitude (± S.E.M.) of the negative (●) and positive (○) waves of averaged field potentials derived during application of glutamate (A) and GABA (B). Field potentials induced by 32 fimbria-fornix stimuli were recorded during continuous superfusion of the septal surface with ACSF. At the instant indicated by an upward arrow, the ACSF was replaced by one of the drugs in ACSF. Administration of the drugs was terminated at the moment indicated by the downward arrow. Examples of averaged field potentials recorded at 3 moments during the experiment with glutamate (A) and GABA (B) are displayed at the right of the diagrams.

DISCUSSION

In the present study, only part of the LSC neurons examined were excited by iontophoretically administered AVP. In the majority of the LSC cells though, the peptide induced a marked increase in the excitatory responses to GLU, ASP, QUIS and NMDA which frequently lasted for several minutes after termination of the peptide administration. In addition, we found that the peptide-induced increase in excitatory amino acid responses was accompanied by a similar increase in the excitations evoked in LSC cells by transsynaptic stimuli. These results confirm and extend the previously obtained data on the action of AVP in the LSC[9,10], though are in contrast with data reported by others[14].

According to extensive biochemical[24] and electrophysiological[11,12] evidence, the excitations evoked in LSC cells by fi-fx afferents are mediated by GLU or a related excitatory amino acid. It seems of significance that the presently observed action of AVP consisting of a prolonged increase of the neuronal responsiveness to amino acid induced excitations accompanied by an enhanced responsiveness to fi-fx stimuli probably mediated by an excitatory amino acid is demonstrated in a structure which is a recipient of both vasopressinergic[1,20] and amino acid[24] innervation.

The locus and mechanism involved in the AVP-induced response enhancement can not be determined from the present extracellular study. However, the fact that AVP only affected responses to excitatory amino acids while the responses to GABA remained unaltered in presence of the peptide, seems to indicate that the peptide itself did not induce a depolarizing effect on the membrane potential.

The single unit evidence on the capacity of AVP to increase the synaptic excitatory responses of LSC cells is strengthened by the effect of the peptide observed on the FPs. Evidence has been provided[5,6] that the leading negative and subsequent positive wave in the FPs represent the sum of excitatory and inhibitory postsynaptic activations synchronously triggered in many of the LSC cells by fi-fx stimuli. In the present study, the leading negative wave of the FPs increased markedly during and after topical administration of AVP. Thus, also from these experiments an augmented excitatory response of LSC cells to fi-fx stimuli is indicated. The positive wave of the FPs showed no consistent changes after the peptide administration suggesting that the inhibitory responses in the LSC neurons are presumbly much less susceptible to the action of the peptide.

The AVP-induced increase in the negativity without apparent changes in the positivity of the FPs differed markedly from the action of GLU, GABA and muscimol on the FPs. Topically administered GLU reduced both the negative and positive waves of the FPs. It is well known that GLU excites the majority of the neurons in the mammalian brain and spinal cord by depolarization of these neurons[4,25]. The decrease in the negative wave of the FP can therefore be explained by the GLU-induced depolarization of the LSC cells. The presently observed decrease in the positive wave of the FP was most probably due to an indirect restriction of the inhibitory input to the LSC cells. It has been postulated that the inhibition elicited in LSC cells by fi-fx stimuli is mediated via a negative feedback loop activated by the excitation of LSC cells[7,14]. Consequently, the extent to which inhibition is evoked in LSC neurons depends on the 'amount' of excitation in these cells. Cells depolarized by GLU will not elicit an action potential in response to fi-fx stimuli and thus will not contribute to the feedback inhibitory input.

GABA and muscimol inhibit the mammalian neurons by hyperpolarization of the neurons[17]. The magnitude of the inhibitory postsynaptic potentials will decrease. The marked decrease of the positive waves of the FPs observed during superfusion of the septum with GABA and muscimol are in agreement with this interpretation.

Since the selective change in the negative wave of the FPs induced by AVP does not resemble the action of either the depolarizing or hyperpolarizing agents described above, it seems that the action of the peptide does not involve similar changes in the membrane potentials as the other compounds.

Recently it was demonstrated that single periferal osmotic stimuli induced in 15 min a release of 30–50 pg of AVP from the LSC of rats[18]. In the present study, we demonstrated a noticeable increase in responsiveness of LSC cells to excitatory synaptic stimuli already with ca. 1 pg of the peptide (10 μl of 10^{-10} M AVP). The observed action of AVP could thus be of physiological significance. Based on the

present results we therefore postulate that the inducement of a prolonged increase in the responsiveness to excitatory amino acid input may be one of the functions of AVP in the LSC and perhaps also in other brain structures that are a recipient of both vasopressinergic and amino acid innervations.

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