Patch-Clamp Analysis of Spontaneous Synaptic Currents in Supraoptic Neuroendocrine Cells of the Rat Hypothalamus

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Spontaneous synaptic currents were recorded in supraoptic magnocellular neurosecretory cells using whole-cell patch-clamp techniques in the rat hypothalamic slice preparation. Numerous spontaneous excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) were observed in the 27 cells recorded. The rate of occurrence of the spontaneous currents and the relative proportion of EPSCs versus IPSCs varied significantly from cell to cell. Miniature EPSCs and IPSCs were clearly distinguished from background noise in TTX ($n = 3$ cells at $0.5 \mu\text{g/ml}$). The frequency of EPSCs and IPSCs decreased by approximately 70% and the largest events were blocked in TTX, but the peaks of the amplitude histograms were shifted by only a few picoamperes. Bicuculline ($n = 10$ cells at $10 \mu\text{M}$ and 2 cells at $20 \mu\text{M}$) blocked completely all the IPSCs without any detectable effect on the frequency or amplitude of the EPSCs. No slow spontaneous outward currents, indicative of a K^+ current from activation of GABA_A receptors, were observed. The α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate-type glutamate receptor antagonist 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX; $n = 7$ cells at $10 \mu\text{M}$) consistently blocked all EPSCs without any apparent effect on the frequency or amplitude of the IPSCs. No synaptic events could be detected when CNQX was applied in combination with bicuculline ($n = 4$). The decay phase of averaged spontaneous IPSCs and EPSCs recorded at resting membrane potential could be well fitted by single exponential functions in most cells. The time constants ranged from 0.92 to 3.0 msec for EPSCs (five cells) and from 5.3 to 6.6 msec for IPSCs (four cells). A second, slower time constant of 4–15 msec was found in the largest averaged EPSCs ($\geq 40 \text{ pA}$). The amplitude of this slow component was -2 to -4 pA . These results suggest that, in the *in vitro* slice preparation, glutamate mediates all the spontaneous EPSCs in magnocellular neurosecretory cells by acting primarily on AMPA/kainate-type receptors at resting membrane potential and that activation of GABA_A receptors mediates most if not all IPSCs.

[Key words: hypothalamus, supraoptic nucleus, neurosecretion, magnocellular neuroendocrine cells, amino acids, synaptic currents, patch clamp]

Magnocellular neuroendocrine cells of the supraoptic nucleus have long been considered as a model system for a wide range of studies on neurosecretion. These neuroendocrine cells synthesize the neuropeptide hormones oxytocin and vasopressin and transport them, from their cell bodies in the supraoptic and paraventricular nuclei, along their axons to the neurohypophysis where they are secreted directly in the general circulation. The supraoptic nucleus has particular advantages for electrophysiological studies on neuroendocrine cells because, unlike other hypothalamic nuclei, virtually all of these cells project to the neurohypophysis and are therefore neuroendocrine. Numerous substances (e.g., ACh, norepinephrine, opioid peptides, dopamine, histamine, 5-HT, substance P, vasopressin, oxytocin, cholecystokinin, somatostatin) have been proposed to regulate or modulate hypothalamic neurosecretion (for review of the neuropharmacology of this system, see Renaud and Bourque, 1991). A wide range of studies on conventional neurons, as opposed to hypothalamic neuroendocrine cells, suggest that these substances primarily serve as neuromodulators rather than neurotransmitters responsible of traditional synaptic potentials. Until recently, however, relatively few studies have directly tested the hypothesis that excitatory and inhibitory amino acids (i.e., glutamate and GABA) mediate all fast synaptic transmission in the magnocellular neuroendocrine system (Gribkoff and Dudek, 1988, 1990; Wuarin and Dudek, 1991). Recent evidence suggests that most of the excitatory and inhibitory synapses, not only in the magnocellular system, but throughout the neuroendocrine hypothalamus may be glutamatergic and GABAergic, respectively (van den Pol et al., 1990).

Studies using intracellular recordings showed that two broad-spectrum antagonists for excitatory amino acids, kynurenic acid and γ -D-glutamylglycine, inhibit electrically evoked and spontaneous EPSPs in the rat supraoptic nucleus (Gribkoff and Dudek, 1988, 1990). Most of the electrically induced excitatory synaptic input to magnocellular and parvocellular neurons in the paraventricular nucleus and to neurons in the arcuate nucleus has been shown to be inhibited or blocked by 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX), suggesting that glutamate mediates excitatory synaptic events mainly by activating AMPA/kainate-type receptors (van den Pol et al., 1990; Wuarin and Dudek, 1991). Immunohistochemical studies have shown glutamate-reactive presynaptic boutons in contact with dendrites and cell bodies in the supraoptic nucleus (Meeker et al., 1989; van den Pol et al., 1990). Therefore, recent electrophysiological and immunohistochemical observations have provided evidence that glutamate is a neurotransmitter at many excitatory synapses in the supraoptic nucleus and elsewhere in the hypothalamus.

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Figure 1. Whole-cell patch-clamp recording of a supraoptic magnocellular neuroendocrine cell with both excitatory and inhibitory inputs. *A–C* are data from the same cell. *A*, Spontaneous currents recorded at resting membrane potential (-66 mV); R_{in} was 700 M Ω . Traces are continuous. *B* and *C*, Histograms showing distribution of the EPSC and IPSC amplitudes, respectively. Current amplitudes were measured during 6 min epochs from data such as shown in *A*. Note that in this cell there were approximately four times more EPSCs than IPSCs.

A recent anatomical study using post-embedding surface staining with immunogold showed that GABA-immunoreactive terminals compose approximately 50% of the synaptic input, not only to the supraoptic nucleus, but also to paraventricular, arcuate, and suprachiasmatic nuclei. This observation strongly suggests that GABA is the most important inhibitory neurotransmitter in the magnocellular system and in the hypothalamus (Decavel and van den Pol, 1990). Intracellular recordings from supraoptic neurons obtained in the explant preparation showed an abundance of spontaneous IPSPs that were chloride dependent and mediated by GABA_A receptor activation (Randle et al., 1986). Extracellular stimulation in the diagonal band of Broca evoked IPSPs that closely resembled the spontaneous events. These results suggest that supraoptic magnocellular neurons receive an extensive and powerful inhibitory GABAergic input, originating at least in part in structures located rostral to the supraoptic nucleus.

The electrophysiological studies mentioned above used sharp microelectrodes for intracellular recording. This technique allows detection of spontaneous EPSPs but has limitations regarding the resolution of small events. Synaptic potentials mediated by substances other than glutamate or GABA may be relatively small and may not have been detected. Another po-

tential problem of these previous studies is the use of extracellular electrical stimulation to evoke synaptic responses. The stimuli were delivered through electrodes positioned near the supraoptic or paraventricular nucleus, a location that gave large and consistent synaptic responses. With this method, however, it is likely that only a fraction of the entire input to a nucleus is activated, and that afferent fibers releasing neurotransmitters other than amino acids were not stimulated. In the present study, we used whole-cell patch-clamp recordings in conventional hypothalamic slices (Blanton et al., 1989) to address the two problems mentioned above. This technique provides a signal-to-noise ratio far superior to intracellular recording with sharp electrodes and allows detection of miniature synaptic events (Edwards et al., 1990; Otis et al., 1991). Testing the effects of amino acid receptor antagonists on spontaneous EPSCs and IPSCs would be expected to provide a more rigorous assessment of the importance of the excitatory and inhibitory amino acids (i.e., glutamate and GABA) as transmitters for the neuroendocrine cells in the supraoptic nucleus.

The aims of this study were (1) to establish the properties of spontaneous postsynaptic currents in supraoptic magnocellular neuroendocrine cells; (2) to determine if glutamate, acting on AMPA/kainate-type receptors, mediates all detectable excitato-

Figure 2. Comparison of the rate of spontaneously occurring synaptic events in two different cells. Traces in *A* and *B* show two samples (5 sec) of spontaneous synaptic activity at a relatively slow time scale. Upward deflections were IPSCs and downward deflections were EPSCs. Frequency histograms of IPSCs and EPSCs (during 115 sec) are shown for each cell. The average rate for IPSCs and EPSCs was, respectively, 4.2 sec⁻¹ and 13 sec⁻¹ for the cell in *A* and 0.7 sec⁻¹ and 1.1 sec⁻¹ for the cell in *B*.

ry synaptic currents; (3) to test if GABA_A receptors mediate all detectable IPSCs; and (4) to determine if activation of NMDA receptors contributes to the decay of EPSCs. The properties and components of the EPSCs and IPSCs were examined through analyses of the time course of the events, and the role of AMPA/kainate and GABA_A receptors was evaluated with bath application of CNQX and bicuculline.

Materials and Methods

Slice preparation. Immature male rats (50–80 gm) were decapitated, and their brains rapidly dissected and placed in ice-cold perfusion solution (see below) for 1 min. During the dissection, particular care was taken to cut the optic nerves without applying any tension on them. A block of tissue containing the hypothalamus was then trimmed and glued on the stage of a vibrating microtome. Usually two slices of 400–600 μ m containing the supraoptic nucleus were cut in a frontal plane and immediately transferred in a ramp chamber (Haas et al., 1979) oxygenated with 95% O₂, 5% CO₂, and thermoregulated at 34°C. The perfusion solution contained (in mM) 124 NaCl, 26 NaHCO₃, 3 KCl, 1.3 MgSO₄, 1.4 NaH₂PO₄, 2.4 CaCl₂, and 10 glucose; pH 7.4. The following drugs were applied in the perfusion solution: bicuculline methiodide (10 and 20 μ M), TTX (0.5 μ g/ml) (both from Sigma, St. Louis, MO), and CNQX (3 and 10 μ M, from Tocris Neuramin, Buckhurst Hill, England).

Recording methods. Whole-cell current recordings (Hamill et al., 1981) were obtained using patch pipettes with open resistances of 2–5 M Ω . Seal resistances were 1–10 G Ω ; series resistances were 5–25 M Ω com-

pensated at >80%. Patch pipettes were filled with (in mM) 140 K-glucuronate, 10 HEPES, 1 NaCl, 1 CaCl₂, 1 MgCl₂, 5 BAPTA, and 4 ATP (pH 7.2). ATP was used to decrease the rundown of IPSCs. Without 4 mM ATP, IPSCs gradually disappeared within the first 20 min (Stelzer et al., 1988). The pipettes were pulled from borosilicate glass capillaries of 1.7 mm diameter and 0.5 mm wall thickness. Currents were recorded using an Axopatch-1D amplifier, low-pass filtered at 2 kHz, digitized at 44 kHz (Neuro-Corder, Neurodata Inc.), and stored on videotapes. The amplitude and decay phases of spontaneous synaptic currents were measured on a personal computer using pCLAMP programs (Axon Instruments) with a sampling rate of 10 kHz. Spontaneous currents were recorded at resting membrane potential, which was less negative than the reversal potential for IPSPs (Randle et al. 1986); therefore, inward currents were considered as EPSCs and outward currents as IPSCs.

Cell labeling. Six cells were recorded with pipettes containing biocytin (0.2%, $n = 2$; 0.4%, $n = 4$). At the end of the experiment, the slice containing the marked cell was immersed in 4% paraformaldehyde in 0.1 M sodium cacodylate buffer overnight. The tissue was then cut at 50 μ m and rehydrated. The sections were labeled with an avidin-biotinylated horseradish peroxidase reaction (Horikawa and Armstrong, 1988). To ascertain that the labeled cells were within the boundaries of the supraoptic nucleus, the slices were counterstained with cresyl violet.

Results

Recordings were obtained from 27 cells in 23 slices. Once the whole-cell configuration was established, stable recordings were achieved for several hours without noticeable change in resting

Figure 3. Whole-cell recording of a magnocellular neuron showing predominantly IPSCs. This cell showed bursts of IPSCs separated by periods of relative inactivity. *A*, Example of a burst of IPSCs. Between bursts, the presynaptic cell was presumably silent (see top and bottom two traces). Traces are continuous. *B*, Amplitude histogram of IPSCs from cell shown in *A* measured during a period of approximately 8 min. *C*, Frequency of spontaneous IPSCs during a period of 2 min 20 sec, including two bursts of IPSCs. The second peak corresponds to the burst shown in *A*. Recording was at resting membrane potential (-60 mV); R_{in} was 900 M Ω .

membrane potential or input resistance. Mean resting membrane potential was -63 ± 2.5 mV (mean $\pm$ SE, $n = 21$), and input resistances ranged from 400 to 1100 M Ω with a mean of 726 ± 50 M Ω (mean $\pm$ SE, $n = 25$). All recordings were made with the holding potential set to the same value as the resting membrane potential for the recorded cell.

Although it was relatively easy to position recording electrodes within the supraoptic nucleus under visual control using slices cut in the frontal plan, we also recorded from six cells with pipettes containing biocytin in order to label them and verify that we were indeed recording from magnocellular neurons. This confirmed that we were not recording from cells at the periphery of the nucleus that have been hypothesized to be GABAergic neurons (Theodosis et al., 1986). We recovered four cells, and they were located well within the boundaries of the supraoptic nucleus (two cells could not be found in the stained sections).

Numerous spontaneous synaptic events were observed in all cells. Inward currents were defined as EPSCs and outward cur-

rents as IPSCs (Fig. 1). The IPSC amplitudes ranged from 10 to 80 pA, and the average amplitude for each cell was between 15 and 30 pA. The EPSC amplitudes ranged from -10 to more than -150 pA, and the average amplitude was from -10 to -50 pA. The rate of occurrence of spontaneous EPSCs and IPSCs varied from <1 Hz to >10 Hz (Fig. 2). Three cells showed bursts of spontaneous IPSCs, and the maximum frequency of IPSCs during a burst was >20 Hz (Fig. 3). The proportion of EPSCs versus IPSCs also varied from cell to cell; four cells showed mostly IPSCs, and two cells showed only EPSCs.

Effects of TTX on synaptic currents

We applied TTX in the perfusion solution at 0.5 μ g/ml to test if synaptic activity could be detected after action potentials had been blocked (Fig. 4). In the three cells tested, miniature EPSCs and IPSCs could be clearly distinguished from baseline noise. Figure 5 shows an example of amplitude distributions of spontaneous EPSCs and IPSCs before and during the application of TTX.

Figure 4. Comparison of spontaneous synaptic activity in normal perfusion solution (*top*) and 20 min after addition of 0.5 $\mu\text{g/ml}$ TTX (*bottom*). The cell R_{in} was 500 M Ω . Traces are continuous, and holding potential was the same as resting membrane potential (-55 mV).

Effect of bicuculline and CNQX on spontaneous synaptic currents

To determine if all the IPSCs were due to the activation of GABA_A receptors, we tested bicuculline on 10 cells at 10 μM and on 2 cells at 20 μM . Bicuculline, at both concentrations, blocked completely all the IPSCs in all the cells tested. This block was reversible (Fig. 6). Bicuculline had no effect on the frequency and amplitude of EPSCs and it did not modify input resistance or resting membrane potential.

In order to test the hypothesis that all spontaneous EPSCs in magnocellular neuroendocrine cells are mediated by glutamate, we applied the non-NMDA receptor-selective antagonist CNQX in the perfusion solution. The antagonist was tested on six cells at 10 μM and on one cell at 3 μM . In the seven cells tested, CNQX reversibly blocked all the EPSCs. In three cells, bicuculline (20 μM) was applied before CNQX to block IPSCs and isolate the EPSCs. When CNQX was then added to bicuculline in the perfusion solution, no synaptic activity could be detected (Fig. 7).

Time course of decay phase of spontaneous currents

Decays of spontaneous currents were measured from averaged EPSCs and IPSCs recorded at resting membrane potential. The synaptic currents were averaged by using the first half of the rising phase to align them. Both EPSCs and IPSCs could be fitted by a single exponential function (Fig. 8). The fit was quite good for all the averaged IPSCs. Decays of the smaller averaged EPSCs (i.e., ≤ 30 pA) could be well fitted by a single exponential ($\tau = 2.4 \pm 0.38$ msec; $n = 4$; e.g., Fig. 8, cells 1, 3, and 4). However, in four of the larger averaged EPSCs (≥ 40 pA;

Figure 5. Histograms of the amplitudes of EPSCs (*left*) and IPSCs (*right*) recorded from the cell shown in Figure 4 in normal solution and in TTX-containing solution. In each condition, EPSCs and IPSCs were measured during a 1 min period. The *solid columns* represent the amplitudes of EPSCs and IPSCs recorded in the presence of 0.5 $\mu\text{g/ml}$ TTX. Note that the peak of the EPSC amplitude histogram was decreased only from -9 to -8 pA by TTX. The peak of the IPSC amplitude distribution was decreased from -13 to -10 pA.

e.g., Fig. 8, cells 2 and 5), the decay was better fitted by the sum of two exponentials. The second time constant second was 4–15 msec, and its amplitude was -2 to -4 pA. No significant difference was found in decay time constant of averages of the smallest and the largest synaptic currents (Fig. 9).

Discussion

Patch-clamp recordings of spontaneous currents versus previous studies

The present study used whole-cell patch-clamp recordings in the “thick slice” (Blanton et al., 1989) to investigate the electrophysiology of the magnocellular system. All the previous studies of neurotransmission in the magnocellular system used sharp microelectrodes, which provide relatively low signal-to-noise ratio. The necessity of using particularly high-resistance electrodes to obtain stable recordings in magnocellular neuroendocrine cells compounded the problem of the low resolution of this type of electrode and made it difficult to record spontaneous events routinely. Only high-quality recordings allowed clear detection of spontaneous synaptic activity (Randle et al., 1986; Gribkoff and Dudek, 1990). The previous work was also based mostly on the study of electrically evoked synaptic responses. The rationale for this approach was to obtain responses that were large enough for accurate measurement of the effects of selective antagonists. After averaging, this allowed analysis of dose–response relations (Wuarin and Dudek, 1991). One of the weaknesses of the electrical stimulation technique is that one can only activate a particular pathway(s), and therefore evoked synaptic responses may not reveal all of the afferent inputs to a cell. Considering these limitations and the amount of evidence accumulated over the years in favor of other neurotransmitters in the magnocellular system, we reexamined the importance of glutamatergic and GABAergic neurotransmission in the magnocellular system by studying spontaneous synaptic events with a technique that allows greatly superior resolution over sharp microelectrodes.

The improved signal-to-noise ratio provided by patch pipettes allowed us to detect spontaneous miniature synaptic currents

Figure 6. In bicuculline, no IPSCs were detected. *Upper panel* shows recording under control conditions. *Middle panel* shows the effect of bath-applied bicuculline (15 min at 10 μ M). The IPSCs recovered from the block, but only after more than 90 min of washout (*bottom panel*). All traces are continuous, holding potential was -70 mV throughout the experiment, and cell R_{in} was 1 G Ω .

in all the cells tested with TTX. The amplitude of the miniature EPSCs and IPSCs was well above noise level in each cell tested. It is therefore reasonable to assume that we would have been able to detect any spontaneous EPSCs and IPSCs not mediated by glutamate or the activation of GABA_A receptors when the non-NMDA receptor antagonist CNQX and bicuculline were present in the perfusion solution.

It has been shown that spontaneous GABA- and glutamate-mediated synaptic currents can be observed in an isolated mammalian central neuron (Drewe et al., 1988). These currents were Ca²⁺ dependent and blocked by γ -D-glutamylglycine and bicuculline. These results suggest that the presence of functional synaptic boutons attached to the postsynaptic cell is a sufficient condition for miniature spontaneous activity to occur, and that the cell bodies and the axons of the projecting cells are not necessary. Therefore, spontaneous activity in the slice may provide a qualitative means to study all of the inputs to a given cell.

One of the major limitations of the patch-clamp technique is the dialysis of the cell cytoplasm. The consequence is "run-down," which affects mainly currents dependent on the activation of G-protein-coupled receptors. Ligand-gated channels generating fast events, such as the nicotinic ACh receptor, seem to be less susceptible to washout. We did not detect any obvious

Figure 7. No spontaneous EPSCs or IPSCs were detected in the presence of bicuculline and CNQX. As shown in Figure 6, no IPSCs were observed in bicuculline (20 μ M; *upper panel*). When CNQX (10 μ M) was added to the perfusion solution in the presence of bicuculline (20 μ M), all spontaneous synaptic currents were blocked (*middle panel*). The effect of CNQX could be reversed after approximately 120 min (*bottom panel*). Cell R_{in} was 600 M Ω ; holding potential was resting membrane potential (-65 mV). Traces are continuous.

decrease in the spontaneous activity within the first 3 min of recording, which suggests that rapid washout of synaptic currents after establishment of the whole-cell configuration did not occur. However, we found that Mg²⁺ in the intracellular solution was necessary to avoid washout of all IPSCs within 30 min. When Mg²⁺ was present in the pipette, we did not notice any obvious decrease in the frequency of spontaneous events, even over a period of several hours of recording. The fact that EPSCs and IPSCs recovered from the effect of CNQX and bicuculline, respectively, argues against the possibility that washout occurred during application of the antagonists.

EPSCs and glutamate

The results obtained in the present study support and extend the earlier work showing that kynurenic acid, γ -D-glutamylglycine, and CNQX inhibit evoked EPSPs in supraoptic and paraventricular magnocellular neuroendocrine cells (Gribkoff and Dudek, 1988, 1990; Wuarin and Dudek, 1991). As mentioned above, these studies used extracellular electrical stimulation to evoke synaptic responses and high-resistance sharp microelectrodes with relatively low resolution. Although these results

Figure 8. Decay phase of IPSCs and of most EPSCs was well fitted by single exponential functions. Traces are averaged spontaneous EPSCs and IPSCs from five different cells (1–5) obtained at resting membrane potential in normal solution. Cell 4 did not show any IPSCs. The time constant of the fit (broken line) is indicated for each averaged current. The number of averaged events is shown by *n*. Cell 5 is the same cell as shown in Figure 2A; however, only EPSCs were averaged. Traces in 5a and 5b are identical (note different calibration for cells 1–4 and cell 5). The decay phase of cell 5 was poorly fitted with a single exponential function (5a). The sum of two exponential functions (5b) shows a small component (–4 pA) with a relatively slow time constant (τ_s) and a larger component (–34 pA) with a fast time constant (τ_f).

showed that glutamate is the major excitatory neurotransmitter in the magnocellular neuroendocrine system, the methods used did not allow one to exclude an important role of other neurotransmitters. There is an abundance of data showing that other neuroactive substances influence vasopressin and oxytocin secretion as well as increase the firing activity of magnocellular neurons. Among the most studied potential neurotransmitters with excitatory actions in the magnocellular system are ACh, norepinephrine, dopamine, histamine, cholecystokinin, and angiotensin (Renaud and Bourque, 1991). In other systems in the brain, these substances have been shown to have slow effects. In the magnocellular system, evidence has been presented suggesting the existence of a monosynaptic cholinergic input onto supraoptic neurons (Hatton et al., 1983). As described above, the signal-to-noise ratio obtained with patch pipettes would have allowed us to detect any small events mediated by neurotransmitters such as ACh or histamine (Yang and Hatton, 1989). We did not detect any inward current in the seven cells tested with CNQX. The present results support the hypothesis

Figure 9. EPSCs and IPSCs of different amplitudes had similar decay phase time constants. Traces are averages of spontaneous IPSCs (top traces, cell 1) and EPSCs (bottom traces, cell 2) recorded at resting membrane potential in the control perfusion solution. The broken lines superimposed on each trace represent the fit of the decay phase of the synaptic current to a single exponential function. For each averaged current, time constant of the fit of the decay phase is indicated. The number of events per averaged trace is indicated by *n*. For each cell, averages of *n* of the largest and of the smallest postsynaptic currents are shown on the left and right columns, respectively. Spontaneous IPSCs (cell 1) were from the same cell as shown in Figure 3. Cell 2 had a resting membrane potential of –58 mV, and its input resistance was 700 M Ω .

that glutamate probably mediates all the fast EPSCs in magnocellular neuroendocrine cells.

NMDA receptors and EPSCs

Several recent studies have demonstrated that NMDA receptors are present in supraoptic cells. A DL-2-amino-5-phosphonopentanoic acid (AP-5)-sensitive component to electrically evoked postsynaptic potentials could be revealed in the slice preparation when Mg^{2+} was deleted from the perfusion solution (Gribkoff, 1991). The amplitude of excitatory synaptic potentials evoked by electrical stimulation of the organum vasculosum lamina terminalis was increased in Mg^{2+} -free medium and inhibited by AP-5 (Yang and Renaud, 1991). Depolarization (Hu and Bourque, 1991a) and rhythmic bursting activity (Bourque and Hu, 1991; Hu and Bourque, 1991b) could be induced in magnocellular neurons by application of NMDA. These results suggest that NMDA receptors are present on these cells, that they influence their firing properties, and that they play a role in synaptic transmission.

In hippocampal granule cells (Keller et al., 1991), NMDA-dependent evoked EPSCs recorded in cells voltage clamped near resting membrane potential had a decay that could be fitted by a double exponential whose time constants were approximately 1 and 2 orders of magnitude slower than that of the non-NMDA component. In the thin cerebellar slice, evoked EPSCs in granule neurons had a slow component that could be blocked by AP-5. Miniature EPSCs were composed of an initial fast non-NMDA component followed by clearly discernable NMDA channels (Silver et al., 1992). Averaging of these miniature currents revealed that the channel openings formed a second slower synaptic component. The amplitude of the slow NMDA component, in both spontaneous and induced EPSCs, was approximately 10% of the fast component amplitude. To detect

a potential NMDA component in the spontaneous EPSCs at resting membrane potential, we measured the time course of the decay phase using exponential functions. We found that the decay phase of averaged spontaneous EPSCs in magnocellular neuroendocrine cells could be well fitted with one exponential in most cells. In a few cells, however, we found a second component characterized by a small amplitude (approximately 10% that of the fast component) and a time constant 5–10 times slower than the fast time constant. This slow phase of the decay of spontaneous EPSCs is reminiscent of an NMDA component, such as described in the studies mentioned above. Since most of the channels linked to the NMDA receptors are blocked at resting membrane potential by the Mg^{2+} present in the extracellular medium (Mayer et al., 1984; Nowak et al., 1984; Jahr and Stevens, 1990), NMDA currents are expected to be relatively small at that potential. Possible space-clamp problems might have allowed the largest EPSCs to depolarize the membrane potential sufficiently to allow NMDA receptors to be activated; however, since no significant difference was found in decay time constant of averages of the smallest and the largest EPSCs, the decay of the EPSCs was probably not determined by cell membrane passive properties (Llano et al., 1991). Taken together with results obtained using extracellular electrical stimulation in the paraventricular nucleus (Wuarin and Dudek, 1991), our results do not support the notion of a significant activation of NMDA receptors in spontaneous EPSCs of supraoptic magnocellular neurons at resting membrane potential. This contrasts with results obtained in cerebellar granule cells (Silver et al., 1992), hippocampal granule cells (Keller et al., 1991), and neocortical pyramidal cells (Wuarin et al., 1992) where a more consistent NMDA component to the excitatory synaptic responses was found. Future experiments designed to evaluate the voltage dependence of spontaneous EPSCs are needed for a more complete evaluation of the role played by the NMDA receptors in synaptic transmission in the magnocellular neuroendocrine system.

GABA and IPSCs

GABA is considered to be the dominant inhibitory neurotransmitter in the magnocellular system (van den Pol, 1985; Jhamandas and Renaud, 1986; Randle et al., 1986; Renaud and Bourque, 1991) and throughout the hypothalamus (Decavel and van den Pol, 1990). However, other neuroactive substances have been shown to inhibit vasopressin and oxytocin secretion and to decrease the firing rate of magnocellular neuroendocrine cells. Examples are norepinephrine (Armstrong et al., 1982; Arnaud et al., 1983) and opioid peptides (Wuarin and Dudek, 1990). Using the same strategy that we used for the EPSCs, we searched for small or relatively slow events, once all the fast GABA_A receptor-mediated currents were blocked by bicuculline. We did not detect any outward currents in the 12 cells tested with the GABA_A receptor antagonist bicuculline. To our knowledge, there is no report of spontaneous GABA_B receptor-mediated synaptic currents. Although it is possible that these currents could have been washed out immediately after obtaining the whole-cell recording configuration, no studies using sharp electrodes have presented evidence for spontaneous inhibitory synaptic events mediated by GABA_B receptors. These results support the hypothesis that all IPSCs, at least all fast IPSCs, are mediated by activation of GABA_A receptors.

The time constants of spontaneous IPSCs were faster than previously reported for spontaneous IPSPs measured in the hy-

pothalamic explant preparation (Randle et al., 1986). This previous study used sharp electrodes and current-clamp recordings, and the decay of spontaneous IPSPs was measured on recordings done with microelectrodes filled with 3 M KCl. The longer time constant of the decay of IPSPs reflects the passive properties of the membrane, which are classically considered to set the slow decay of IPSPs.

Source of spontaneous currents

Studying spontaneous synaptic currents has advantages over electrically evoked responses for assessing the overall synaptic input to a cell. In the *in vitro* slice preparation, however, several factors are likely to influence spontaneous synaptic activity. Many afferent axons are cut during the preparation of the slice; all the afferents fibers from other parts of the brain and brainstem and from hypothalamic regions (Swanson and Sawchenko, 1983) not contained in the slice are obviously cut. Axons from some local cells that project to the supraoptic nucleus through a trajectory outside the plane of the slice are also cut. Part of the strong GABAergic input (van den Pol, 1985) to the supraoptic nucleus has been proposed to originate from GABA neurons located around the nucleus itself (Theodosis et al., 1986); therefore, GABAergic neurons projecting to supraoptic cells may still be present in the slice.

The striking differences that we observed between cells in terms of the frequency of events and the proportion of EPSCs versus IPSCs may be the result of different cells having different local input. The largest EPSCs and IPSCs were most likely due to action potentials originating from cut axons or from local circuits containing the soma of the projecting cell and its axon. The bursts of IPSCs that we observed in some cells are likely to be caused by bursts of action potentials in a GABAergic cell projecting to the recorded cell. Therefore, GABAergic inhibitory and glutamatergic excitatory local circuits probably exist and are functional in the slice.

Conclusions

The concept of neurosecretion was established four decades ago (Scharrer and Scharrer, 1954), and the supraoptic magnocellular neuroendocrine cells are the classic model of neurosecretory cells. Possible difference between conventional neurons and neuroendocrine cells in terms of passive membrane properties and synaptic transmission has been a long-standing question (Finlayson and Osborne, 1975; Mason and Bern, 1977; Yagi and Iwasaki, 1977). In the present study, the identification of neuroendocrine cells was unequivocal because we used direct visual identification of the supraoptic nucleus, and staining of cells showed that we were recording from cells in the nucleus. As reviewed by Renaud (1987, 1991), the intrinsic and synaptic properties that have been described in neuroendocrine cells are similar or identical to what is known to occur in neurons. Our observations on spontaneous EPSCs and IPSCs also support the hypothesis that neuroendocrine cells integrate electrical and chemical signals similarly to regular neurons. In particular, the present results support the hypothesis that, in the *in vitro* slice preparation, (1) glutamate mediates all the fast spontaneous EPSCs to magnocellular neuroendocrine cells, and (2) GABA acting on GABA_A receptors mediates all the fast spontaneous IPSCs. The other neuroactive amines and peptides proposed as neurotransmitter candidates in the magnocellular neuroendocrine system are perhaps not neurotransmitters such as defined in the neuromuscular junction (Katz, 1969), but rather neuro-

modulators. By changing cell membrane potential and input resistance over long periods of time, they influence directly the amplitude and duration of the fast synaptic currents and therefore indirectly modulate the secretion of oxytocin and vasopressin.

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