Effect of Adrenalectomy on Miniature Inhibitory Postsynaptic Currents in the Paraventricular Nucleus of the Hypothalamus

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Verkuyl, J. M. and M. Joëls. Effect of adrenalectomy on miniature inhibitory postsynaptic currents in the paraventricular nucleus of the hypothalamus. J Neurophysiol 89: 237–245, 2003; 10.1152/jn.00401.2002. Within the rat paraventricular nucleus of the hypothalamus two types of neurons have been distinguished based on morphological appearance, i.e., parvocellular and magnocellular neurons. The parvocellular neurons play a key role in regulating the activity of the hypothalamo–pituitary–adrenal axis, which is activated, e.g., after stress exposure. These neurons receive humoral negative feedback via the adrenal hormone corticosterone but also neuronal inhibitory input, either directly or transsynaptically relayed via GABAergic interneurons. In the present study we examined to what extent the neuronal GABAergic input is influenced by the humoral signal. To this end, miniature inhibitory postsynaptic currents (mIPSCs) were recorded in parvo- and magnocellular neurons of adrenalectomized rats, which lack corticosterone, and in sham-operated controls. Under visual control neurons in coronal slices containing the paraventricular nucleus were designated as putative parvocellular or magnocellular neurons: the former were located in the medial part of the nucleus and displayed a small fusiform soma; the latter were mostly located in the lateral part and were recognized by their large round soma. Compared with putative magnocellular neurons, parvocellular neurons generally exhibited a lower membrane capacitance, lower mIPSC frequency, and smaller mIPSC amplitude. Following adrenalectomy, the mIPSC frequency was significantly enhanced in parvo- but not magnocellular neurons. Other properties of the cells were not affected. In a second series of experiments we examined whether the increase in mIPSC frequency was due to the absence of corticosterone or caused by other effects related to adrenalectomy. The data support the former explanation since implantation of a corticosterone releasing pellet after adrenalectomy fully prevented the change in mIPSC frequency. We conclude that, in the absence of humoral negative feedback, local GABAergic input of parvocellular neurons in the paraventricular nucleus is enhanced. This may provide a compensatory mechanism necessary for maintaining controllable network activity.

INTRODUCTION

Within the rat paraventricular nucleus of the hypothalamus (PVN) two types of neurons have been distinguished based on morphological appearance, i.e., parvocellular and magnocellular neurons. Parvocellular neurons are key regulators of the hypothalamus–pituitary–adrenal (HPA) activity. Thus HPA activity is driven by corticotropin-releasing hormone (CRH) and cosecretagogues released from the parvocellular neurons. CRH causes the release of adrenocorticotropic hormone from the pituitary, which in turn stimulates the secretion of corticosterone from the adrenal cortex (see Whitnall 1993). Corticosterone induces peripheral effects but also feeds back to the PVN to inhibit, via glucocorticoid receptors, CRH synthesis and release, thus indirectly downregulating its own secretion (Swanson and Simmons 1989). In addition to affecting the PVN, corticosteroids also influence other brain areas, such as the hippocampus and amygdala (De Kloet et al. 1998).

The setpoint of HPA activity is not only determined by the humoral feedback via corticosterone but also by neuronal signals integrated in the PVN. The PVN receives excitatory inputs from several brain areas, such as the amygdala (Feldman and Weidenfeld 1998), the dorsomedial hypothalamus (Morin et al. 2001), and several brain stem areas (see Herman and Cullinan 1997). However, the PVN also receives a dense inhibitory input. About 50% of the hypothalamic synapses are GABAergic (Decavel and Van den Pol 1990). Part of these involve direct GABAergic projections to the PVN, originating, e.g., in the suprachiasmatic nucleus (Hermes and Renaud 1993) and arcuate nucleus (Cowley et al. 1999). Other areas, such as the cingulate cortex (Diorio et al. 1993) and hippocampus (Herman et al. 1994)—also enriched in corticosteroid receptors—transsynaptically inhibit the PVN via hypothalamic interneurons located among others in the bed nucleus stria terminais and peri-PVN regions (Boudaba et al. 1996; Roland and Sawchenko 1993; Tasker and Dudek 1993). Nearly all CRH parvocellular neurons express GABA receptors (Cullinan 2000), underpinning the importance of neuronal input in suppressing PVN and thus HPA activity (Herman and Cullinan 1997; Herman et al. 2002).

Since parvocellular neurons in the PVN receive humoral as well as neuronal feedback signals, it is conceivable that these two pathways do not work independently. This is supported by pharmacological studies in which either the humoral or neuronal feedback signal was blocked. For instance, injection of the GABAA receptor antagonist bicuculline close to the PVN caused an increase of CRH, vasopressin, and c-FOS expression in the paraventricular subregion of the PVN and increased circulating corticosterone levels (Cole and Sawchenko 2002). Conversely, removal of the humoral feedback by adrenalecto-
tomy led to increased benzodiazepine binding, as measured in whole-hypothalamus preparations of the rat. This effect was reversed by corticosteroid substitution (De Souza et al. 1986; Goeders et al. 1986; Majewksa et al. 1985).

We here addressed the question to what extent the local GABAergic network in the PVN adapts if the humoral feedback signal, i.e., glucocorticoid input, is dysfunctional. To this end rats were adrenalectomized (ADX), allowing the investigation of neuronal feedback in the absence of corticosteroids. To monitor neuronal feedback at the synaptic level, miniature inhibitory postsynaptic currents (mIPSCs) were recorded with the whole-cell patch-clamp technique. Frequency, peak amplitude, and kinetic properties of mIPSCs in PVN neurons were compared in tissue from ADX and sham-operated control rats. Reintroduction of corticosterone in ADX rats was used to show steroid dependence of changes after ADX.

METHODS

Surgery and slice preparation

Thirty-eight male Wistar rats (Harlan CBP, Horst, The Netherlands) of 90–190 g were housed under standard conditions and received food, water, and saline (ADX) ad libitum. Day/night fluctuations of hormones of interest were standardized by a constant light/dark cycle (08:00–20:00/20:00–08:00 h). All experiments were approved by the local Animal Experiment Committee (DEC project No. DED43). Three days before the experiment at 09:30 h, rats were bilaterally adrenalectomized (n = 12) or sham operated (n = 14) under halothane (Sano, Sante, Netherlands) anesthesia as described earlier (Ratka et al. 1989). In a second series of experiments, ADX rats (n = 8) received a subcutaneous 25-mg corticosterone pellet (Innovative Research of America), which is known to result in moderately high circulating levels of corticosterone (Ratka et al. 1989). Control ADX rats (n = 4) received a placebo pellet.

On the day of the experiment at 09:00 h, rats were placed in a novel environment (clean cage) for 30 min after which they were quickly decapitated. Trunk blood was collected for determination of plasma corticosterone by RIA. The brain was quickly removed from the skull and decapitated. Trunk blood was collected for determination of plasma corticosterone by RIA. Due to manipulation before decapitation, all animals were decapitated for determination of circulating corticosterone. The brain was quickly removed from the skull and decapitated. Trunk blood was collected for determination of plasma corticosterone by RIA. Due to manipulation before decapitation, all animals were decapitated for determination of circulating corticosterone.

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Recordings and analysis

An upright microscope with a 40× water immersion objective and 10× ocular was used to identify PVN neuron subtypes based on their location and the shape of their cell body. Whole-cell voltage-clamp recordings were made using an Axopatch 200B amplifier (Axon Instruments). Patch pipettes were pulled from borosilicate glass (Science Products, Holheim, Germany) on a horizontal puller (Sutter Instruments). The pipettes were filled with an intracellular buffer containing (in mM) 140 CsCl, 10 HEPES, 10 EGTA, 2 MgATP, and 0.1 NaGTP (all from Sigma); pH adjusted with CsOH (Acros Organics, Geel, Belgium) to 7.2; 280 mOsm; pipette resistance 4–7 MΩ. For later off-line visualization a limited number of cells was filled with either Lucifer yellow (4 mg/ml; Molecular Probes, Leiden, The Netherlands) or Alexa Hydrozin 488 (1.75 mM; Molecular Probes). Series resistance and capacitance were monitored during the whole recording using pCLAMP7 (Axon Instruments). Recordings with an uncompensated series resistance of less than 2.5 times the pipette resistance were accepted for analysis.

Traces of 5 min were recorded using the gap-free acquisition mode of pCLAMP7 at a 10-kHz sampling rate. mIPSCs were detected off-line using CDR and WCP analysis software (J. Dempster, University of Strathclyde, Glasgow, UK, http://www.strath.ac.uk/Departments/PhysPharm/ser.htm (2002 Feb. 25)), which uses a threshold-based event detection algorithm. Of all mIPSCs the inter-mIPSC interval, rise time, peak amplitude, and τ of decay were determined. The decay of each mIPSC was fitted with a mono- and bieponential curve in WCP. This program uses the Levenberg–Marquardt algorithm to iteratively minimize the sum of the squared differences between the theoretical curve and the data curve. WCP indicates the goodness of fit with the SD of the residuals between the fitted curve and the data points (residual SD) for each mIPSC fitted. As criterion for the goodness of the fit the residual SD should be <0.2. Fitting with a bieponential instead of a monoexponential curve did not increase goodness of the fit since it did not decrease the residual SD as tested in a substantial number of cells. Also there was no significant change in the variance of the residual of the monoexponential compared with the variance of the residual of the bieponential, as tested with a Student’s t-test for a random sample of individual mIPSCs of both putative parvocellular and magnocellular neurons (n = 17). We chose to fit with the function using the least number of parameters, i.e., the monoexponential.

Microsoft Excel was used to select individual mIPSCs of each cell with the following criteria: 1) peak amplitude should be larger than 10 pA; 2) rise time, taken as 10 to 90% of peak amplitude should be <5 ms; 3) the τ of the decay time based on a monoexponential fit should be between 4 and 50 ms. These criteria are based on earlier studies, describing mIPSC properties in other hypothalamic nuclei or other brain areas (Brussaard et al. 1997; Wierenga and Wadman 1999). Based on these criteria about 14% of all initially mIPSC detected events were discarded, equally distributed over the different treatment groups. After this analysis, averages of the mIPSC parameters were determined per cell. Also, mIPSC frequency was calculated by dividing the number of events by the recording time in seconds. In addition to averaging the mIPSC parameters per cell, we also analyzed the distribution of mIPSC interval, peak amplitude, and τ of decay in all cells. Frequency distribution per cell for the inter-mIPSC interval was fitted with a exponential curve $y = A_o \exp(-rt)$, with $r$ representing the mean of the intervals. The log of the peak amplitude (Borst et al. 1994) and τ of decay distributions were fitted with a Gaussian curve $y = A_o \exp(-(x-\mu)^2)/\sigma^2$, where $\mu$ represents the mean and $\sigma$ the SD. The frequency distribution for the capacitance was also fitted with a Gaussian. Due to seasonal fluctuations in (uncontrolled) room temperature the second series of experiments had to be corrected for temperature using the Q10 method. The Q10 was experimentally determined by comparing the ADX groups of the two series. The Q10 for the frequency was found to be 1.92, for peak amplitude 1.32, and for the fitted $\tau$ of decay 0.86. The rise time was not temperature dependent.

Statistical analysis was performed with a two-tailed unpaired Student’s t-test. Differences in variance were tested with a F test. Differences were considered significant if $P < 0.05$.

RESULTS

Identification of paraventricular neurons

Individual PVN neurons (n = 89) were identified based on shape and location of their somata. In the in situ (live, un-

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stained) slice preparation of the hypothalamus, subdivisions of
the PVN were clearly distinguished (Fig. 1, A and B). A medial
cell could be discerned, located between the third ventricle and
a lateral cluster of large neurons. Using 400× magnification,
small and usually fusiform neurons were observed within the
medial part of the PVN, with large neurons scattered in be-
tween. The latter displayed usually large round cellbodies,
similar to the cells in the lateral cluster. A limited number of
cells were stained with the intracellular dyes Lucifer yellow
(n = 5) or Alexa Hydrozin 488 (n = 7). Post hoc histological
analysis of these cells confirmed the location and shape of the
cellbody as established during the recording session (see ex-
amples in Fig. 1, A–D). Since the intracellular dyes were found
to influence the physiological properties of the cells, staining
was only performed in a limited number of cells and not
routinely applied. Only those neurons that could be identified
during the recording session as being either 1) medially located
as well as small and/or fusiform or 2) located in the lateral
cluster with a large and round cell body were included in the
present study. Based on these criteria nine cells were excluded
from further analysis. Further subdivisions as described in the
literature for stained sections (Kiss et al. 1991) could not be
made in the unstained slice preparation. In view of the location
and shape of the somata, the medially located small and fusi-
form neurons will be referred to as putative parvocellular
neurons; large neurons located in the lateral cluster will be
referred to as putative magnocellular neurons (Kiss et al.

These two groups of neurons differed in their basic prop-
ties. Putative parvocellular neurons had a significantly smaller
capacitance than putative magnocellular neurons (Fig. 1E and
Table 1), confirming the visual identification based on cell size.
In this analysis of the two cell groups, data from all hormonal
treatment groups (see following text) were pooled since none
of the treatments affected membrane capacitance significantly
(data not shown). Interestingly, of the 45 recorded putative
parvocellular neurons, 5 exhibited a large capacitance (Fig.
1E), although they were visually identified as a small neuron in
the medial part of the PVN. The capacitance of these putative
parvocellular neurons, i.e., 35, 40, 42, 43, and 43 pF, was
roughly two SDs removed from the mean of this group. The
capacity of these cells was even larger than the mean
capacitance of the putative magnocellular neurons. Except for
their large capacitance, however, these cells did not differ from
the other putative parvocellular neurons with respect to their
mIPSC characteristics and the effect of adrenalectomy (see
following text); they were therefore included in the putative
parvocellular neurons group.

mIPSCs

Of both groups of neurons whole-cell patch-clamp record-
ings at −65 mV were made to study mIPSCs. Since these
recordings were made with approximately equimolar concen-
trations of chloride ions inside and outside, currents reversed at
0 mV (n = 3) (Fig. 2, A and B). These currents could be fully
blocked with bicuculline (n = 3), confirming that they were
indeed generated via activation of GABA_A receptors (Fig. 2C).

The basal mIPSC characteristics of the two neuron groups
were studied in SHAM-operated control rats. With respect to
mIPSC characteristics the two groups of PVN neurons differed
greatly. The mIPSC frequency of putative parvocellular neu-
rons was significantly lower than that of putative magnocel-
lar neurons (Fig. 3, A and B and Table 1). Moreover, mIPSCs
of putative parvocellular neurons displayed a smaller peak
current than mIPSCs of putative magnocellular neurons (Fig. 3,
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parvocellular neurons tended to be slower than seen in mag-
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nocellular cells, as shown for a typical example in Fig. 3C.
average this difference did not attain statistical significance (Table 1).

**Hormonal influences on mIPSC characteristics**

In the first series of experiments we studied the effect of ADX on mIPSC characteristics in the PVN for both groups of neurons. Based on the averaged numbers per cell, ADX significantly increased the mIPSC frequency by 68% in the putative parvocellular neurons (Fig. 4A and C). In addition to averaged mIPSC frequency per cell, the distribution of mIPSC intervals was also analyzed for all parvocellular cells in the ADX and SHAM groups, as shown for representative examples in Fig. 4D. In all cases the interval distribution per cell could be fitted with an exponential curve, indicating that the mIPSCs occurred independently from each other. Moreover, the largely increased values for the constants in the exponential fits (see examples in Fig. 4D) confirm the considerable increase in mIPSC frequency after ADX.

In the group of putative parvocellular neurons, no significant effects were observed after ADX on either peak amplitude or $\tau$ of decay, as calculated from the averages per cell (Fig. 5A and C). The lack of effect was confirmed when the distribution of mIPSCs within individual cells was taken into account. Thus, in all cells except one, the distribution of the lognormal of the peak amplitude and $\tau$ of decay could be described with a single Gaussian curve (for peak amplitude, SHAM: average $r^2 = 0.80 + 0.03$; ADX: $0.87 + 0.02$; for $\tau$ of decay, SHAM: average $r^2 = 0.91 + 0.03$; for ADX: $0.89 + 0.03$; typical examples shown in Fig. 5, B and D). In one cell from the ADX group, a better fit of the distribution of lognormal of the peak amplitude was obtained with a double Gaussian. Importantly, after ADX there was no change in the mean as well as the variances of the distributions (as tested with an $F$ test), indicating that the distributions of the peak amplitude and the fitted $\tau$ of decay were in all respects comparable for the ADX and SHAM groups.

Within the putative magnocellular neurons, ADX resulted in a small but nonsignificant increase of the mIPSC frequency, based on the averaged numbers per cell ($P = 0.27$; Fig. 4, B and D). Similar to what was seen in the putative parvocellular neurons, ADX did not affect peak amplitude or $\tau$ of decay in putative magnocellular neurons (Fig. 5, A and C). Also, the frequency distribution of mIPSC interval in putative magnocellular neurons (representative examples in Fig. 4F) as well as the distribution of the lognormal of peak amplitude and $\tau$ of decay (Fig. 5, B and D) were fully comparable for the ADX and SHAM groups.

In the second series of experiments we investigated whether the effects as seen in putative parvocellular neurons after ADX were caused by the absence of corticosteroids. If so, restoring corticosterone level to that of the SHAM-operated controls should normalize the mIPSC characteristics. To this end, eight ADX rats received a subcutaneous corticosterone pellet (25 mg). Pellet implantation indeed resulted in comparable corticosterone levels ($9.63 + 0.39 \mu g/dl; n = 8$) as observed in SHAM-operated controls ($9.95 + 2.50 \mu g/dl; n = 14$). In this second experimental series, control ADX rats received a placebo pellet. As predicted, the mIPSC frequency of putative parvocellular neurons in the corticosterone-replaced ADX rats was indeed decreased compared with the frequency in rats receiving a placebo pellet ($P < 0.002$; Fig. 6). The temperature corrected mIPSC frequency of ADX rats receiving corticosterone replacement was comparable to that of SHAM rats. Compared with the placebo-treated ADX group, corticosterone replacement also changed the averaged $\tau$ of decay (20%, $P < 0.05$) and peak amplitude (42%, not significant) but these changes were substantially less pronounced than the >150% change in mIPSC frequency.

**DISCUSSION**

**Characterization of PVN neurons**

In this study we investigated to what extent absence of a humoral inhibitory feedback signal influences the local properties of the neuronal inhibitory input to the PVN. To this end, mIPSC characteristics of PVN neurons were compared between SHAM and ADX rats. This influence is particularly relevant for parvocellular PVN neurons, given their key role in the HPA axis activity. As a first step we therefore attempted to distinguish parvocellular from magnocellular neurons, based on the location and morphology of their somata, a criterion also used in earlier immunohistological studies performed in the PVN. Morphological distinction was more straightforward than using electrophysiological criteria earlier found with sharp electrodes (Tasker and Dudek 1991), since the presently used whole-cell recording configuration and pipette solution precluded a meaningful comparison.

The distinction on basis of morphological characteristics appeared to be a reliable approach since putative parvocellular and magnocellular neurons in the PVN on average differed from each other with respect to their basic membrane capacitance and mIPSC properties, in a way that is in accordance with other findings. Thus, in general, parvocellular neurons displayed a lower membrane capacitance than magnocellular neurons, which agrees with the difference in their somatic surface. Interestingly, a limited number of cells within the parvocellular cell group that were visually

### TABLE 1. Capacitance and mIPSC characteristics of putative parvo- and magnocellular neurons

<table>
<thead>
<tr>
<th>Parvocellular</th>
<th>Magnocellular</th>
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<tr>
<td>Capacitance (pF)</td>
<td>20.6 ± 1.3</td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>0.498 ± 0.063</td>
</tr>
<tr>
<td>Peak (pA)</td>
<td>−93.1 ± 16.0</td>
</tr>
<tr>
<td>$\tau$ (ms)</td>
<td>15.0 ± 0.8</td>
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Data are represented as means ± SE. For all visually identified PVN neurons the capacitance differed greatly between putative parvocellular and putative magnocellular neurons. Within the SHAM-operated rats the two groups of PVN neurons also differed in mIPSC characteristics, such as frequency peak amplitude, but not in fitted $\tau$ of decay. $n$ is number of identified cells; PVN, paraventricular nucleus of the hypothalamus; mIPSC, miniature inhibitory post-synaptic currents.
identified as having a small cell soma and were located in the medial part of the PVN had a very large capacitance. Except for their large capacitance, however, these cells do not differ from the other putative parvocellular neurons in their mIPSC characteristics or the effect of adrenalectomy. The large capacitance but small cell soma could indicate that the dendrites, particularly large diameter first order branches, also contribute to the capacitance measurement. We can presently not exclude that this small group of neurons represents a subset of parvocellular neurons that is morphologically different from the majority of cells.

Magno- and parvocellular neurons also differed from each other with respect to the mIPSC characteristics. While mIPSCs in magnocellular neurons displayed a high-frequency, large peak amplitude and fast decay, parvocellular neuron mIPSCs were low in frequency, had a small peak amplitude, and were more slowly decaying. The mIPSC characteristics for magnocellular neurons as found in this study closely resemble those described for magnocellular neurons in the SON (Brussaard et al. 1997). The higher frequency of mIPSCs in magnocellular neurons may be related to the fact that the percentage of inhibitory synapses making contact with the soma is higher in the magnocellular than the parvocellular region, as established with electronmicroscopy (Decavel and Van den Pol 1990). Since the present recordings mostly reflect somatic currents, the high mIPSC frequency of the magnocellular neurons may be caused by the high number of somatic GABAergic synapses on these cells. The difference in peak amplitude or quantal amplitude could point to a difference in the number of postsynaptic GABA receptors.

FIG. 2. Original traces recorded from typical parvocellular neurons showing that mIPSCs are carried by chloride ions and generated by GABA_A receptors. A: recordings were made with equimolar concentrations of chloride ions inside and outside the cell. Therefore the currents reverse at 0 mV and are inward at negative holding potentials. B: IV plot of traces shown in A. C: currents were completely blocked by 10 μM bicuculline (BIC).

FIG. 3. Visually identified PVN neurons differed in their mIPSC characteristics. A: original trace recorded from PVN neurons. Left traces: visually identified putative parvocellular neurons. Right traces: putative magnocellular neurons. Note the difference in peak amplitude and frequency. For more detailed comparison, B shows a blowup of the original trace of a putative parvocellular (left) and magnocellular neuron (right). C: averaged mIPSCs of typical putative parvocellular (solid black line) and putative magnocellular neuron (dashed line). For comparison of the τ of decay, the peak IPSC amplitude of the putative parvocellular neuron was scaled to that of the magnocellular neuron (gray line).
aptic GABA<sub>A</sub> receptors (Nusser et al. 1997). Indications for higher levels of GABAergic receptors in magnocellular than parvocellular neurons come from in situ hybridization studies, showing that the magnocellular section of the PVN consistently exhibits a higher expression of GABA<sub>A</sub> receptor subunits than the parvocellular section (Cullinan and Wolfe 2000). The small difference in mIPSC τ of decay between parvo- and magnocellular neurons may represent differences in synaptic

**FIG. 4.** Effect of ADX on PVN neurons. A: original traces of putative parvocellular neurons. Left traces: SHAM rats. Right traces: ADX rats. Note the increases in mIPSC frequency. B: original traces of putative magnocellular neurons. Left traces: SHAM rats. Right traces: ADX rats. C: histogram showing increased mIPSC frequency in putative parvocellular neurons from ADX rats; the gray bar the averaged mIPSC frequency of putative parvocellular neurons from ADX rats. *Statistically significant at P < 0.01 (unpaired two-tailed Student’s t-test). D: typical examples of distribution of the mIPSC interval (bin size 1 ms) from a putative parvocellular neuron of a SHAM rat (left) and an ADX rat (right). Distributions were fitted with an exponential curve (solid line). Note higher constants for the cell of the ADX rat. E: adrenalectomy did not affect (P = 0.27) mIPSC frequency of magnocellular neurons. Open bar: averaged (+SE) mIPSC frequency of putative magnocellular neurons from SHAM rats. Light gray bar: averaged mIPSC frequency of magnocellular parvocellular neurons from ADX rats. F: typical examples of distribution of the mIPSC interval (note smaller bin size of 0.25 ms) from a putative magnocellular neuron of a SHAM rat (left) and an ADX rat (right). Both curves were fitted with an exponential curve. Note comparable constants for both groups.
Adrenalectomy did not affect peak amplitude (A and B) or τ of decay (C and D) for both the putative parvocellular neurons and magnocellular neurons. A: histogram showing average (+SE) peak amplitude of putative parvocellular neurons in SHAM (black bar) and ADX rats (gray bar) and putative magnocellular neurons in SHAM (white bar) and ADX (light gray) bar. B: typical example of distribution of the mIPSC peak amplitude for a single putative parvocellular neuron in a SHAM rat (top left) and ADX rat (top right). Representative examples for putative magnocellular are given below. C: histogram showing average (+SE) τ of decay of putative parvocellular cell in SHAM (black bar) and ADX rats (gray bar) and of putative magnocellular neurons in SHAM (white bar) and ADX rats (light gray bar). D: typical example of distribution of the mIPSC τ of decay for a single putative parvocellular neurons in a SHAM (top left) and an ADX rat (top right). Similarly, examples of putative magnocellular neurons are given below.
parameters such as subunit composition, transmitter uptake, or diffusion of GABA in the synaptic cleft (Cherubini and Conti 2001).

**Effect of ADX**

In the HPA system corticosteroids feed back primarily on the PVN, to downregulate HPA activity. This is done in concert with direct or transsynaptic inhibitory inputs to the PVN from higher brain areas and local hypothalamic areas. To investigate to what extent absence of a humoral inhibitory feedback signal in the PVN in ADX influences the local properties of the neuronal inhibitory input to the PVN, the ADX model was selected.

The data show that corticosteroids and the GABAergic innervation indeed do not work independently. Reducing corticosteroids levels by ADX increased the mIPSC frequency of parvocellular neurons; mIPSC frequency of magnocellular neurons—which are not directly involved in the HPA system—was not altered, indicating a specific effect on the GABAergic system involved in stress. Restoring corticosterone levels in ADX rats reduced mIPSC frequency of parvocellular neurons to SHAM level, emphasizing that the effect of ADX is indeed due to the absence of corticosterone. Tau of decay was also slightly but significantly changed when comparing ADX rats receiving corticosterone to ADX rats receiving a placebo. Perhaps this difference can be explained by the fluctuating corticosterone levels seen in SHAM rats versus the rather constant and moderately high levels of corticosterone in ADX rats receiving corticosterone via a pellet.

The increase in local GABAergic transmission after ADX is supported by earlier pharmalogical studies. In whole hypothalamic preparations several groups showed increased agonist binding to the benzodiazepine receptor complex after ADX. This effect could be reversed by corticosteroid substitution (De Souza et al. 1986; Goeders et al. 1986; Majewska et al. 1985). Recently, Miklos and Kovacs (2002) found that 7 days after ADX the number of GABAergic terminals specifically on CRH-positive neurons was significantly increased, as established with electron microscopy. The latter observation indicates that the increased mIPSC frequency seen in our study most likely reflects an increase in the number of GABAergic terminals, rather than an increase in release probability. This change in synaptic innervation after ADX could take place in several ways. Thus corticosterone could affect synaptic contacts directly in the PVN. Corticosterone may also act at the level of the limbic structures projecting to the PVN, thus indirectly affecting synaptic contacts in the hypothalamus.

What could be the functional meaning of the ADX-induced increase in GABAergic transmission locally in the PVN? In the ADX model, the corticosteroid feedback signal—which normally downregulates HPA activity—is no longer present. It was shown that the lack of feedback leads to increased levels of CRH secretagogues, in particular CRH and vasopressin (de Goeij et al. 1993; Sawchenko 1987). In brain slices, Kasai and Yamashita (1988) found that the spontaneous firing rate of neurons in the paraventricular region from ADX rats was higher than that of intact rats. In that study, synaptic inputs from other areas were near absent. Apparently, the intrinsic firing rate of parvocellular neurons is increased after ADX. We here show...
that the local synaptic inhibition, however, is increased. Generally, GABAergic innervations are thought of as being important for synchronizing neuronal activity. The increased mIPSC frequency might therefore provide a compensatory mechanism necessary for maintaining controllable network activity.

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