The paraventricular nucleus (PVN) is the major controller of hypothalamus-pituitary-adrenal axis activity, as well as the site of glucocorticoid negative feedback. The catecholaminergic inputs from the brainstem that provides the major excitatory drive on the activity of the PVN cells. In addition, the dense GABAergic inputs provides the major inhibitory transmission to the PVN, which are originated mostly from the extra-nuclear regions, particularly the peri-PVN areas. The local GABAergic inputs to the PVN are modulated by noradrenaline as well as glucocorticoids, but the effect of glucocorticoid on noradrenergic modulation of GABAergic transmission to the PVN are not well known. To understand the actions of glucocorticoids on the synaptic transmission in the neurosecretory neurons of the PVN, in this work, we examined the firing activity of putative neurosecretory PVN neurons, and the effects of noradrenaline on GABAergic transmission in adrenalectomized rats. We also characterized the electrophysiological and morphological properties of the GABAergic neurons in peri-PVN region by using the GAD65-eGFP transgenic mice.

Experiment I: Effect of adrenalectomy on noradrenergic modulation of GABAergic transmission in neurosecretory neurons of the PVN. In this experiment, we examined the effects of noradrenaline on the frequency of inhibitory postsynaptic current (IPSC) in the neurosecretory neurons of the adrenalectomized rats. The neurosecretory PVN neurons were labeled by a retrograde fluorescent dye (DiI) injected into the pituitary stalk and further classified to type I, or type II by electrophysiological properties. Noradrenaline reduced the frequency of spontaneous IPSCs in most type I and about half of type II neurons, but enhanced the frequency in the rest of type I and type II neurons.

The bilateral adrenalectomy increased the proportion of neurons showing the noradrenergic reduction in the IPSC frequency in type II neurons, and the extent of the reduction in both type I and II neurons. A corticosterone supplement (10 mg pellet) reversed the adrenalectomy-induced changes. BRL44408, a β2A-adrenoceptor antagonist blocked the noradrenergic reduction in type II PVN neurons of the adrenalectomized rats, but not tertodotoxin. The immunoreactive particles of ??-adrenoceptors in the PVN were more numerous in adrenalectomized than in the sham-operated rats. The majority of ??-adrenoceptor immunoreactivities were closely associated with synaptophysin or GAD65 immunoreactivities. In addition, the mRNAs of corticotrophin-releasing hormone and vasopressin were detected from the labeled type II PVN neurons. Collectively, the results suggest that depletion of corticosterone by adrenalectomy markedly potentiated the noradrenergic suppression of GABAergic transmission that is mediated by the β2A-adrenoceptors located on the GABAergic terminals in the neurosecretory PVN neurons. The results imply that noradrenaline can more efficiently increase the excitability of neurosecretory cells in the absence of glucocorticoid negative feedback by potentiating noradrenergic disinhibition of GABAergic transmission in the PVN.

Experiment II: Effect of adrenalectomy on the excitability of neurosecretory parvocellular neurons in the PVN. In this experiment, we examined the effects of adrenalectomy on neuronal excitability at cell-attached current clamp mode. Adrenalectomy increased the frequency of spontaneous firing of action potentials, and induced a burst pattern that was absent in the sham-operated rats. Adrenalectomy also increased the proportion of neurons showing ?1-adrenoceptor mediated noradrenergic excitation. Overall, these results suggest that removal of corticosterone by adrenalectomy can elevate the neuronal excitability by increasing the spontaneous firing rate and by potentiating the ?1-adrenoceptor-mediated noradrenergic excitation, which could facilitate the release of hypothalamic hormones from the neurosecretory PVN neurons.

Experiment III. Properties of GABAergic neurons in subparaventricular zone of the hypothalamus (SPVZ) of GAD65-eGFP transgenic mice. GABAergic inputs make up ~50% of the synaptic innervation of the PVN neurons and under the regulation of noradrenergic inputs from the brainstem. The majority of the GABAergic synaptic inputs to PVN originate from local sources including SPVZ. In this experiment, we examined the electrophysiological and chemical properties of GABAergic
neurons and its noradrenergic modulation in the SPVZ of GAD65-eGFP transgenic mice. Among 81 eGFP neurons tested, five subtypes were observed: burst-spiking (BS, 41%), regular-spiking (RS, 32%), late-spiking (LS, 11%), gap-spiking (Gap, 11%) and single-spiking neurons (SS, 5%). The majority of neurons (68%) showed silent in cell-attached mode. Chemically, dense immunoreactivities were observed for calbindin and calretein (spcheck) in the SPVN, but not for parvalbumin. Furthermore, bath application of noradrenaline (1-100 ?M) depolarized the majority of neurons tested (21/28, 75%), hyperpolarized 3 neurons (11%), and induced no effect in 4 neurons (13%). NA-induced depolarization was concentration-dependent, and was not affected by tetrodotoxin (0.5 ?M, n = 4). Taken together, the present results indicate that there are, at least, five types of GABAergic neurons with distinct electrophysiological and morphological properties, and the noradrenergic inputs induced depolarizing effect on the majority of GABAergic neurons in the SPVZ. The results may provide experimental evidence for noradrenergic increase in the frequency of IPSCs shown in the first experiment.

Experiment IV. Properties of GABAergic neurons in the rostral zona incerta (ZIr) of GAD65-eGFP transgenic mice. The electrophysiological and morphological properties of GABAergic cells in the ZIr adjacent to the PVN were investigated by combining in vitro patch-clamp recording and biocytin-filling techniques in coronal brain slices of a GAD65-eGFP transgenic mouse strain. Five groups of GABAergic cells in ZIr were defined according to their firing pattern: burst-spiking (BS, 42%), regular-spiking with fast afterhyperpolarization (RSfAHP, 25%), regular-spiking with slow afterhyperpolarization (RSsAHP, 20%), late-spiking (7%) and single-spiking (7%) neurons. All LS cells and the majority of BS cells exhibited time-dependent or time-independent inward rectification, but few of RSsAHP cells and none of RSfAHP and SS cells. In cell-attached voltage-clamp mode, the majority of cells (~ 71%) presented either spontaneous tonic regular or irregular firing activity. Morphological analysis revealed that BS neurons were mainly multipolar-shaped and had wide-field projections; RSfAHP neurons were bipolar or bitufted-shaped and had local projections; RSsAHP neurons were multipolar-shaped and had wide-field projections; LS neurons were mainly multipolar-shaped and had medium local projections and SS neurons were bipolar-shaped and had local projections. Finally, we found that BS or RSsAHP cells in the ZIr project to the PVN, the anterior hypothalamus, the dorsal thalamus and the contralateral side of the ZIr. The results indicate that the GABAergic neurons in the ZIr constitute a heterogeneous neuronal population with distinctive electrophysiological and morphological properties, and may provide tonic inhibitory inputs the hypothalamus including the PVN.

In conclusion, the results suggest that the depletion of corticosterone by adrenalectomy may enhance the excitability of neurosecretory PVN neurons by potentiating the noradrenergic disinhibition of inhibitory GABAergic transmission via presynaptic ¥á2A-adrenoceptors (Experiment I), and the noradrenergic enhancement of firing activity via ¥á1-adrenoceptors (Experiment II). The results also indicate that adrenalectomy also can enhances the excitability of neurosecretory cells, in the absence of noradrenergic influence, by increasing the frequency of spontaneous firing and inducing a burst firing. The location of the ¥á1-adrenoceptors and mechanism of induction of burst firing are good subjects of further study.

The properties of GABAergic neurons in the SPVZ (Experiment III) and the ZIr indicate (Experiment IV) that these regions are composed of 5 types of distinct GABAergic neurons and the main types are BS and RS neurons in the SPVZ, and BS neurons in the ZIr. The observations that GABAergic neurons in the SPVZ are mostly silent and depolarization by noradrenaline suggest that the SPVZ is the somatic origin of the IPSCs enhanced by noradrenaline in the neurosecretory PVN cells (Experiment I). Thus, these results provide novel examples of glucocorticoid-induced plasticity in synaptic transmission at neurosecretory PVN neurons and would help to further understand the direct effect of glucocorticoid negative feedback occurring within and/or around the PVN.
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