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Muscimol Inhibits ADH Release Induced by Hypertonic Sodium Chloride in Rats

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IOVINO, M., G. DE CARO, M. MASSI, L. STEARDO AND S. POENARU. *Muscimol inhibits ADH release induced by hypertonic sodium chloride in rats.* PHARMACOL BIOCHEM BEHAV **19**(2) 335–338, 1983.—The effect of the GABA-agonist muscimol on ADH release induced in rats by administration of hypertonic sodium chloride solutions was studied by means of intracerebroventricular and intraperitoneal injections of the drug. Injected by the intracerebroventricular route, muscimol produced a significant reduction of plasma ADH concentration not only in animals treated with hypertonic sodium chloride, but also in unstimulated animals. Following intraperitoneal administration larger doses were required to produce such an effect, thus suggesting a central site of action for the effect of muscimol on ADH release. Bicuculline, given intraperitoneally before muscimol injection, completely blocked ADH inhibition induced by muscimol, thus suggesting a specific involvement of GABAergic receptors. These findings indicate that GABAergic mechanisms may be involved in the regulation of body fluids in the rat by affecting ADH release.

Muscimol Bicuculline Hypertonic sodium chloride ADH release Rat

ACCORDING to classical hypotheses [3, 11, 31] osmo- and/or sodium-receptors, localized in or close to the hypothalamo-neurohypophyseal system, regulate thirst and vasopressin (ADH) release. An acute elevation in cerebrospinal fluid (CSF) sodium chloride concentration, by intracerebroventricular administration of hypertonic sodium chloride solution, stimulates water intake [3, 22, 26, 28, 32], antidiuresis [28,31], secretion of ADH [26,28] and firing rate of neurosecretory neurons in supraoptic (SON) and paraventricular (PVN) nuclei [1]. All these effects are due to the interaction with cerebral receptors sensitive to changes in osmotic pressure [18,22] and/or in CSF sodium concentration [3,22].

Experiments carried out to elucidate whether vasopressinergic neurons themselves are sensors of plasma osmolality or sodium concentration, have suggested that the vasopressinergic neurons are not themselves the sensors [13,18].

At present, the nature of the synapses involved from the osmo- and/or sodium-receptors to the vasopressinergic neurons, as well as the nature of neurotransmitters acting on the vasopressinergic neurons, is not well defined. On the basis of pharmacological experiments, acetylcholine and nicotine have been shown to release ADH from the hypothalamo-neurohypophyseal system [14], and nicotine-cholinergic blocking agents have been demonstrated to inhibit ADH release in response to acetylcholine [24] and osmotic stimulation [25]. In contrast to the excitatory effects of cholinergic agents, adrenergic agents, such as noradrenaline, inhibit the release of ADH [5,19]. Thus vasopressinergic neuron cell membranes have excitatory nicotinic [5, 8, 19, 20] and inhibitory β-adrenergic receptors [4, 5, 19, 20, 23].

As far as GABA is concerned, there is some evidence about its involvement in ADH release. Biomedical studies have revealed the presence in high concentration of GABA and its synthetizing enzyme, glutamic acid decarboxilase (GAD), in the SON and PVN [6, 12, 25, 30]. Moreover, peptidergic axons of the hypothalamo-neurohypophyseal tract are endowed with receptors for GABA [33] and GABA was selectively accumulated in glial cells (pituicytes) in control and denervated neurohypophysis [17]. Furthermore, GABA microiontophoretically applied, depresses the excitability of most SON and PVN neurosecretory neurons [20,21]. Finally, it has been reported that GABA antagonists stimulate secretion of ADH in cats [10].

In the present study, the effect of the GABA agonist muscimol on osmotically stimulated ADH release has been investigated. In addition, the relationship between the effects of intraperitoneal (IP) or intracerebroventricular (ICV) administration of muscimol on ADH release has been examined.

METHOD

General Protocol

Male Wistar rats weighing 200–250 g were housed in an animal room maintained at a constant temperature (23±1°C)

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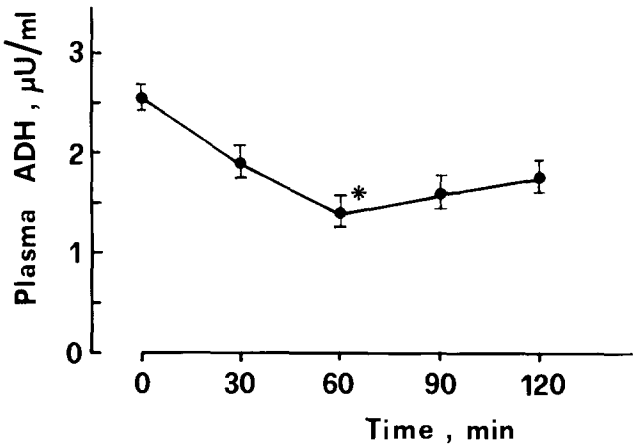

FIG. 1. Time course of plasma ADH concentration following IP administration of muscimol (2 mg/kg). Values are means±S.E.M. of 7–8 data. Difference from control level (time 0): *$p<0.01$; where not indicated, difference was non statistically significant.

TABLE 1

EFFECT OF IP MUSCIMOL (M) ON PLASMA ADH CONCENTRATION INDUCED BY SC HYPERTONIC SODIUM CHLORIDE SOLUTION (HS)

Pretreatment		Treatment	Plasma ADH (μU/ml)	No.
HS	+	—	8.62 ± 1.2	8
HS	+	IS	8.45 ± 1.3*	8
HS	+	M	5.06 ± 0.6†	8
HS + BIC	+	M	8.75 ± 1.3‡	7

Plasma ADH concentration was stimulated by 1 M NaCl (HS) administered SC (15 ml/kg) 3 hr prior to water access. Muscimol (M; 2 mg/kg) or 0.9% NaCl (IS) were administered IP 10 min prior to water access. Bicuculline (BIC; 2 mg/kg) was given IP 6 min prior to muscimol administration. Rats were decapitated 60 min later and ADH extracted from 1 ml plasma was measured by radioimmunoassay. Data represent means ± S.E.M.

*Non statistically different from HS.

†$p<0.02$ vs. HS or HS+IS.

‡Non statistically different from HS.

and humidity (70%), with automatic light control from 06.00 a.m. to 20.00 p.m. Commercial rat chow and tap water were available ad lib.

A stainless steel cannula was stereotaxically implanted, under sodium pentobarbital anaesthesia (Nembutal, IP, 40 mg/kg), into the left lateral cerebroventricle and was fixed to the skull with screws and acrylic dental cement. The ventricular cannula placement was verified by ICV injection of Fast Green dye at the end of the experiment. Only data from rats showing dye distribution in the lateral, third and fourth ventricles were used. The animals were allowed at least 5 days to recover from surgery before being tested.

Drugs

For ICV injections, the GABA agonist muscimol (Sigma Chemical Co., St. Louis, MO) was dissolved in 5 μl of artificial CSF. The CSF solution contained 152.5 mEq/l of sodium ions and had an osmolality of 292 mOsmol/kg water.

For IP injections muscimol as well as the GABA antagonist bicuculline methochloride (Sandoz Produkte, Basel) were dissolved in 0.5 ml of isotonic saline (IS).

Protocol for ADH Secretion

Rats were housed in individual metabolic cages to measure water intake and urine output. Daily water intake and urine output were measured, at the same time of the day (09.00 a.m.), before experiments were commenced, in order to assess pre-injection salt-water balance.

The effect of muscimol on plasma ADH concentration was investigated in the following ways:

(a) IP administration of muscimol. Rats received hypertonic saline (HS) (1 M NaCl) subcutaneously (15 ml/kg) at 09.00 a.m. and water was immediately withheld for 3 hr. This protocol results in thirst and ADH release stimulated by cellular dehydration. Muscimol (2 mg/kg) or isotonic saline (IS) (0.9% NaCl) were administered IP 10 min prior to water access. Plasma ADH concentration was determined 60 min after water presentation.

(b) ICV injection of muscimol. In a second group of rats, ADH release was stimulated by ICV injections of HS (1 M NaCl, 10 μl). Control animals received 10 μl of artificial CSF. Fifteen seconds before this treatment, artificial CSF (5 μl) without or with addition of muscimol (5 μg) was given by the ICV route to the same animals. Water was immediately available after the ICV injections. Plasma ADH concentration was measured 60 sec after the last ICV injection.

To check the specific involvement of GABAergic receptors, in some experiments the GABA antagonist bicuculline (2 mg/kg) was administered IP 6 min before the ICV or IP administration of muscimol.

ADH Radioimmunoassay

Plasma ADH levels were measured according to the method of Kamoi and Hama [16]. The antibody against ADH was produced by repeated injections to rabbits of synthetic arginine-vasopressin coupled with bovine serum albumin (BSA) by glutaraldehyde [29]. Cross reactivity with oxytocin and arginine^8-vasotocin were minimal (0.03% and 0.1%, respectively). Synthetic vasopressin (Sigma Chemical Co., St. Louis, Mo) was used as standard and for iodination. The assay had a minimum sensitivity of 0.08 μU/tube. Coefficient of variation within assay and between assays ranged from 8.4 and 9.4%.

Statistical Analysis of Data

All the results are expressed as the mean±S.E.M., and statistical comparisons were made by Student's t-test.

RESULTS

Effect of IP Muscimol on ADH Release

Plasma ADH concentration in unstimulated rats was 2.6±0.35 μU/ml. When muscimol (2 mg/kg) was injected IP to control rats, plasma ADH concentrations determined at 30, 60, 90 and 120 min after muscimol administration were respectively 1.9±0.18, 1.4±0.25, 1.6±0.2 and 1.75±0.22 μU/ml of plasma (Fig. 1).

The maximum decrease observed at 60 min after the IP administration of muscimol proved to be statistically significant ($p<0.01$).

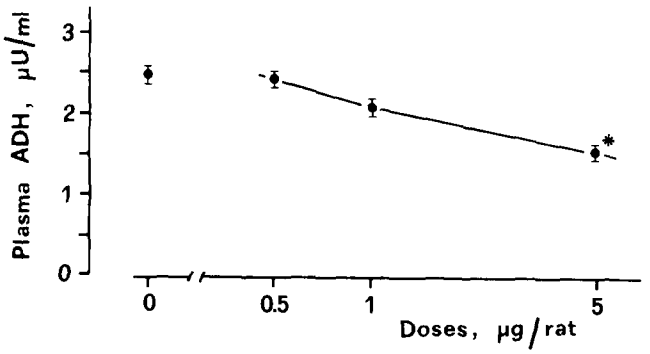

FIG. 2. Effect of ICV injections of 0 (simple isotonic saline), 0.5, 1 or 5 μg of muscimol on plasma ADH concentration. Values are means±S.E.M. of 7–8 data. Difference from controls (dose 0): *$p<0.01$; where not indicated, difference was non statistically significant.

In rats in which ADH release was stimulated by the subcutaneous (SC) administration of HS (1 M NaCl, 15 ml/kg), plasma ADH level was 8.62±1.2 μU/ml. In these conditions IP muscimol (2 mg/kg) again produced a clear-cut reduction of plasma ADH levels (Table 1). In fact rats tested 60 min after muscimol administration showed a plasma ADH concentration of 5.06±0.6 μU/ml, which was statistically lower than that of controls ($p<0.02$). On the other hand, IP administration of IS without muscimol was ineffective (8.45±1.3 versus 8.62±1.2 μU/ml). Bicuculline, given IP 6 min before muscimol injection, completely blocked the effect of muscimol: in fact plasma ADH concentration after bicuculline plus muscimol was 8.75±1.3, which was not significantly different from that of controls.

Effect of ICV Muscimol on ADH Release

Plasma ADH responses to muscimol injected into the lateral cerebroventricle in unstimulated rats are reported in Fig. 2. Trunk blood showed plasma ADH concentration of 2.55±0.25, 2.48±0.28, 2.1±0.2 and 1.63±0.15 μU/ml after the injection of 0 (CSF), 0.5, 1, or 5 μg of muscimol, respectively. The decrease in plasma ADH level following 5 μg of muscimol was statistically significant ($p<0.01$), but the doses of 1 or 0.5 μg of the drug produced no significant effect.

The effect evoked by ICV muscimol (5 μg) was prevented by IP administration of bicuculline (2 mg/kg), as shown in Table 2.

Five μg of ICV muscimol evoked a clear-cut inhibitory effect also when ADH release was stimulated by ICV administration of 10 μl of HS (Table 3). In fact, while control animals receiving ICV HS, but not ICV muscimol, showed a plasma ADH concentration of 10.5±1.05 μU/ml, rats pretreated with muscimol, 5 μg, showed a plasma ADH concentration of 6.8±0.7 μU/ml, which was significantly inferior to that of control animals. Again, bicuculline, given IP 6 min before the ICV injection of muscimol, completely blocked the effect of the latter drug; in fact the observed ADH value (11.3±1.12) was not significantly different from that of controls (10.5±1.05 μU/ml of plasma).

TABLE 2

EFFECT OF ICV MUSCIMOL (M) AND OF ICV MUSCIMOL PLUS IP BICUCULLINE (BIC) ON PLASMA ADH CONCENTRATION

Pretreatment		Treatment	Plasma ADH (μU/ml)	No.
IS	+	CSF	2.75 ± 0.35	7
IS	+	CSF+M	1.47 ± 0.24*	8
BIC	+	CSF+M	2.95 ± 0.35†	7

Bicuculline (BIC; 2 mg/kg) or 0.9% NaCl (IS; 1 ml/kg) were given IP 6 min prior to ICV injection of artificial CSF (5 μl) without or with muscimol (M; 5 μg). Rats were decapitated 60 sec after the ICV injection and ADH extracted from 1 ml plasma was measured by radioimmunoassay. Data are means ± S.E.M.
*$p<0.01$ vs. IS+CSF.
†Non significantly different from IS+CSF.

TABLE 3

EFFECT OF ICV MUSCIMOL (M) ON PLASMA ADH CONCENTRATION STIMULATED BY ICV ADMINISTRATION OF HYPERTONIC SODIUM CHLORIDE SOLUTION (HS)

Pretreatment				Treatment	Plasma ADH (μU/ml)	No.
IP		ICV				
IS	+	CSF	+	HS	10.5 ± 1.05	8
IS	+	CSF+M	+	HS	6.8 ± 0.70*	8
BIC	+	CSF+M	+	HS	11.3 ± 1.12†	7

Artificial CSF (5 μl) with or without muscimol (M; 5 μg) was injected ICV 15 sec prior to 1 M NaCl (HS; 10 μl). Bicuculline (BIC; 2 mg/kg) or 0.9% NaCl (IS; 1 ml/kg) were given IP 6 min prior to ICV injection and ADH extracted from 1 ml plasma was measured by radioimmunoassay. Data represent means ± S.E.M.
*$p<0.01$ vs. IS+CSF+HS.
†Non significantly different from IS+CSF+HS.

DISCUSSION

The results of our experiments show that the ICV administration of muscimol significantly inhibits ADH release both in unstimulated rats and in rats pretreated with hypertonic sodium chloride solution. A similar effect was also elicited by IP administration of muscimol, but doses far larger than those effective after ICV administration were required, thus suggesting a central site of action for the effect of the drug on ADH release.

The observation that the GABA antagonist bicuculline is able to block the inhibitory effect of muscimol, gives strong support in favour of the specific involvement of GABAergic mechanisms in the effect of muscimol on ADH release. In this regard, our findings showing the inhibitory effect of the GABA agonist muscimol are clearly consistent with previous data demonstrating that GABA antagonists evoke ADH release [10]. Moreover, present data showing that muscimol affects basal ADH release and that evoked by HS, taken together with those of a previous study, showing a similar effect of muscimol also on ADH released by angiotensin II [15], suggest a general involvement of GABAergic mechanisms in the control of ADH release.

The results of the present study, while demonstrating a clear-cut effect of muscimol on ADH release, do not give indications on the site of action, nor on the mechanisms by which muscimol exerts such an effect. However some possible mechanisms may be discussed. Firstly, muscimol

may affect sodium- or osmo-sensitive receptors to alter their responsiveness to different sodium chloride concentrations. This effect would be detectable also in rats non stimulated with hypertonic saline, owing to reduction of basal stimuli coming from these receptors to centers involved in ADH release. Secondly, the GABAergic induced reduction of ADH release may be exerted through a presynaptic inhibition in the neural pathways connecting sodium- or osmo-sensitive receptors to cerebral regions involved in body fluid regulation. This hypothesis is supported by the large evidence accumulated demonstrating that GABA is a major neurotransmitter in the vertebrate central nervous system [7]. In this regard, it is noteworthy to mention that high concentrations of GABA and of its synthetizing enzyme, GAD, have been demonstrated in the SON and PVN [6, 12, 27, 30], and that microiontophoretically applied GABA is able to depress the firing rate of most SON and PVN neurons [20,21].

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