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THE VASOPRESSIN AND OXYTOCIN NEUROHYPOPHYSIAL CONTENT AS INFLUENCED BY BLEEDING OR DEHYDRATION: EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE *

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The effect of CCK-8 (50 ng, i. c. v.) on the neurohypophysial vasopressin and oxytocin storage was estimated in haemorrhaged (1 ml per 100 g b. w.) male Wistar rats. In another experimental series rats dehydrated for three days were given CCK-8 in a daily i. c. v. dose of 50 ng. The neurohypophysial vasopressin and oxytocin content was bioassayed by pressor effect following Dekański or milk-ejection activity in vitro following van Dongen and Hays, respectively.
The decrease of neurohypophysial vasopressin and oxytocin content, brought about by dehydration, was significantly less marked in animals treated with CCK-8. The depletion of neurohypophysial vasopressin and oxytocin content in haemorrhaged animals could be completely inhibited by earlier i. c. v. administration of CCK-8. It is suggested that hypothalamic cholecystokinin may serve as a modulator of neurohypophysial function.

Key words: *Cholecystokinin, vasopressin, oxytocin, dehydration, haemorrhage*

INTRODUCTION

Cholecystokinin (CCK) is a polypeptide hormone produced in the upper intestine, but also in the nervous system. Originally isolated as a 33-aminoacid peptide (1), its larger forms have subsequently been characterized extending up to CCK-58 (2). The molecular analysis of the rat brain CCK cDNA showed that CCK was synthetized in the brain tissue as a 115-aminoacid preprohormone (3). The larger molecular forms of CCK (i. e., CCK-33 and CCK-39) probably act *in vivo* as precursors of CCK-8 (4).

There is much evidence now that CCK may serve as a neuromodulator or neurotransmitter in the central nervous system; it has been shown to modify the neuronal function via specific receptors (5, 6). CCK seems to be involved in the regulation of food (7, 8) and water (9) intake as well as in some mechanisms related to reproductive function (10).

High levels of CCK have been measured in the ventromedial hypothalamic nucleus (11) and also in the vasopressin-oxytocin neurosecretory system comprising the posterior lobe of the pituitary as well as the supraoptic and paraventricular hypothalamic nuclei (12). Moreover, CCK is known to be present in oxytocinergic neurons (13). In the paraventricular neurons it coexists with corticotropin-releasing factor, neurotensin, enkephalin, galanin and vasoactive intestinal peptide (14). Nearly 60 per cent of the CCK present in the posterior pituitary lobe is thought to spring from the hypothalamic paraventricular nucleus; the CCK content in the neurohypophysis is markedly depleted under conditions (salt treatment, lactation) known to increase the release of neurohypophysial hormones (15). All these findings clearly suggest that brain CCK may be involved in the control of neurohypophysial function. In fact, previous work (9, 16) showed that chronic treatment with CCK decreases the vasopressin and oxytocin neurohypophysial content.

This report deals with the effects of CCK-8 on the dehydration- and haemorrhage-induced decrease of vasopressin and oxytocin content in the neurohypophysis.

MATERIAL AND METHODS

Animals. The experiments were carried out on a total of 56 adult male Wistar rats weighing 260—330 g. They were fed normal pelleted laboratory diet and kept at about 22°C; a 14-h light, 10-h dark cycle was provided (artificial illumination 6.00 a. m. — 8.00 p. m.). Before experiment they were given tap water *ad libitum*.

The animals were implanted with a permanent cannula inserted into the left cerebral ventricle. Under light hexobarbital anaesthesia, the animals were immobilized in a simple stereotaxic apparatus; a small hole was drilled in the skull (1,5—2,0 mm laterally and 1,5—2,0 mm posteriorly to the crossing of the sagittal and coronal sutures). A simple stainless steel cannula was inserted into the left lateral cerebral ventricle; its tip was 4,0 mm below the dorsal skull surface. The cannula was fixed to the skull with dental cement. After surgery, the animals were allowed to recover for up to 7 days before starting the experimental protocol. The intracerebroventricular (icv) injections were made to previously trained conscious rats; a 50 μl Hamilton syringe with plunger pushed by a microscrew was used.

The effectiveness of i. c. v. infusions was verified indirectly by injecting 10—15 μl of 0,25 per cent trypan blue to similarly operated separate control animals (one rat injected with trypan blue solution for every five injected with CCK-8 solution or vehicle) and was found to be quite satisfactory, i. e. the dye was distributed in an uniform manner within all cerebral ventricles.

The effect of i. c. v. CCK-8 on the vasopressin and oxytocin release following haemorrhage in rats

Experimental design. The rats were divided into two groups:

B — animals treated with cholecystokinin octapeptide (Sigma, NoC-9271, (Tyr-SO$_3$H) — Cholecystokinin, Fragment 26—33) dissolved in sterile saline and administered intracerebroventricularly (i. c. v.) at a dose of 50 ng (i. e., 10 μl of solution), injected at a rate of 10 μl per 30 s;

A — animals similarly injected with 10 μl vehicle (0.9% sodium chloride).

In each group two subgroups were set up:

I — animals injected with CCK or vehicle solution but otherwise not treated; II — animals haemorrhaged (1 ml per 100 g b. w.).

On the day of experiment, the animals were anaesthetized by an intraperitoneal (i. p.) injection of 10% urethane (1,4 ml/100 g b. w.), immobilized on an operating board and the femoral vein was cannulated. Thereafter the course of experiment for subgroups A-II and B-II was as follows:

"O" minute: i. c. v. injection of a solution containing CCK-8 (in group "A") or vehicle (in group "B").

20 th minute: bleeding from the femoral vein (1 ml per 100 g b. w.).

40 th minute: decapitation and immediate removal of the brain with intact pituitary (neurohypophysial extracts preserved for vasopressin and oxytocin determination).

The course of experiment for subgroup A-I and B-I was similar but the animals were not haemorrhaged (i. e., they were injected with CCK-8 or vehicle solution at "O" minute and decapitated at 40 th minute).

The effect of i. c. v. CCK-8 on the vasopressin and oxytocin release during dehydration in rats

Experimental design. The experiment was carried out on animals divided into two groups:

D — animals injected i. c. v. with 10 μl solution containing CCK-8 in a dose of 50 ng (dissolved in 10 μl of 0,9% sodium chloride) once daily (i. e., on "O" time and thereafter following 24, 48, 69 hour, the last dose being administered three hours before killing;

C — animals similarly injected with 10 μl of vehicle solution (0,9% sodium chloride).

In each group two subgroups were set up: I — animals euhydrated and treated once daily with CCK-8 solution or vehicle as described above; II — animals dehydrated for three days and similarly treated with CCK-8 solution or vehicle. The beginning of experiment was considered as "O" time; the animals were killed 72 hours later.

Experimental procedure. The animals were decapitated at 11.00—12.00 a. m.; the brain with intact pituitary was quickly removed and the infundibular stalk cut up. The neurohypopysis was separated within about 3 min and homogenized in 1,0 ml of 0,25% acetic acid in 0,9% sodium chloride. The tissue suspension was transferred into a centrifuge tube and the homogenizer washed with 1 ml of the same solution. The pooled sample was heated for 5 min on boiling water bath, centrifugated for 30 min (room temperature; relative centrifugal force ca 650 g, i. e. ca 6380 m/sec²), the supernatant removed and made up to a constant volume. The extracts were stored at −17°C until assayed for their vasopressor and oxytocic potencies.

Bioassay of vasopressin and oxytocin. The vasopressin (a) and oxytocin (b) content in the neurointermediate lobe were bioassayed by: (a) pressor effect following Dekański (1952; see 17); (milk-ejection activity in vitro following van Dongen and Hays (1966; see 18). Synthetic lysine vasopressin (LVP, Sandoz, Basle, batch No 01214) or synthetic oxytocin (OT, Gedeon Richter, Budapest, batch No 900680489), respectively, were used as reference standard preparations.

For the estimation of vasopressor potency male Wistar rats weighing 300 ± 30 g (mean $\pm$ S. D.) were used as test animals. For each extract to be tested at least one set of four doses (a low dose and a high dose of the standard and unknown, with a dose ratio of $1:2$) was applied and the log potency ratio estimated following (19). The dose of LVP raising the blood pressure by at least 10 mmHg was 0.8—1.2 mU. In our hands, the coefficient of variable calculated following (20) was 13.1%.

For the bioassay of oxytocin primiparous female Wistar rats in midlactation, suckling at least six pups, were used as test tissue donors. As recommended by (21) fifteen mammary cubes were tested for each solution and the mean milk-ejection time was considered to be a quantitative response index of the oxytocic activity. On a double logarithmic scale the milk-ejection time was plotted against appropriate standard dilutions; their relationship being found inverse and linear, the activity of an unknown sample was interpolated. A sensitive test tissue responded to a threshold concentration $10^{-10} - 10^{-8}$ IU/ml. In our hands the coefficient of variable was 3.0%.

Statistical evaluation of the results. The vasopressin and oxytocin content was finally expressed as an equivalent of synthetic lysine vasopressin or synthetic oxytocin for whole neurointermediate lobe. The results are reported as mean $\pm$ standard error of the mean (S. E. M.). For statistical evaluations Student's t-test was used.

RESULTS

The results are summarized on Figs 1—4. Injection of CCK-8, administered to otherwise not treated animals (subgroup B-I; $n = 9$) resulted in no significant changes of the neurohypophysial vasopressin and oxytocin content. The decrease of vasopressin and oxytocin in the neurohypophysis (as brought about by bleeding) was almost completely abolished if haemorrhaged rats were previously treated i. c. v. with CCK-8. In those animals (subgroup B-II; $n = 9$) the actual neurohypophysial vasopressin content was even higher than in respective controls *(Fig. 1 and 2).*

In euhydrated animals treated with CCK-8 over three days (subgroup D-I; $n = 9$) a significant — but not very deep — decrease of both vasopressin and oxytocin in the neurohypophysis could be noted. Under conditions of dehydration the content of both neurohypophysial hormones diminished considerably in the neurohypophysis (subgroup C-II, $n = 9$); if, however, CCK-8 was administered during dehydration, this effect of osmoreceptors' stimulation was almost inhibited (subgroup D-II; $n = 9$): the neurohypophysial contents of vasopressin and oxytocin were not different from those found in CCK-8 — treated, euhydrated animals *(Fig. 3 and 4).*
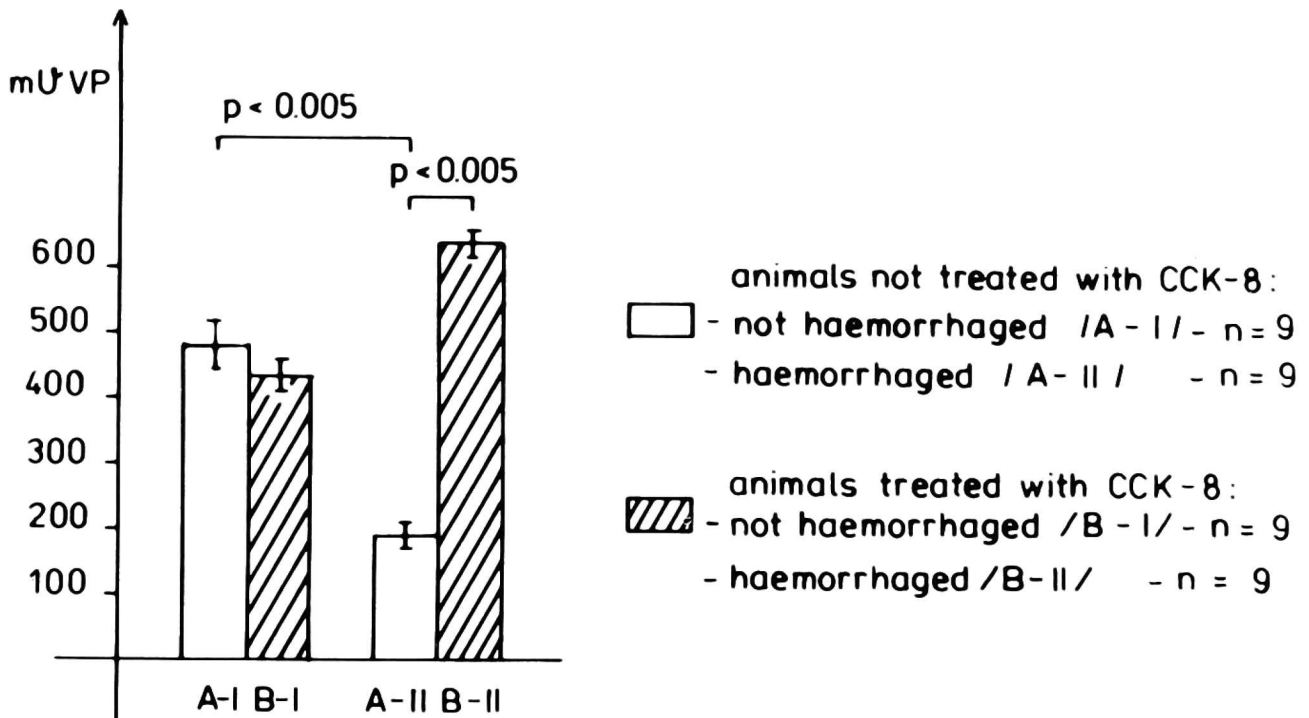
Fig. 1. The neurohypophysial vasopressin content in control and haemorrhaged (1 ml/100 g b. w.) rats following i. c. v. injection of CCK-8 or normal saline (mean ± S. E. M.).
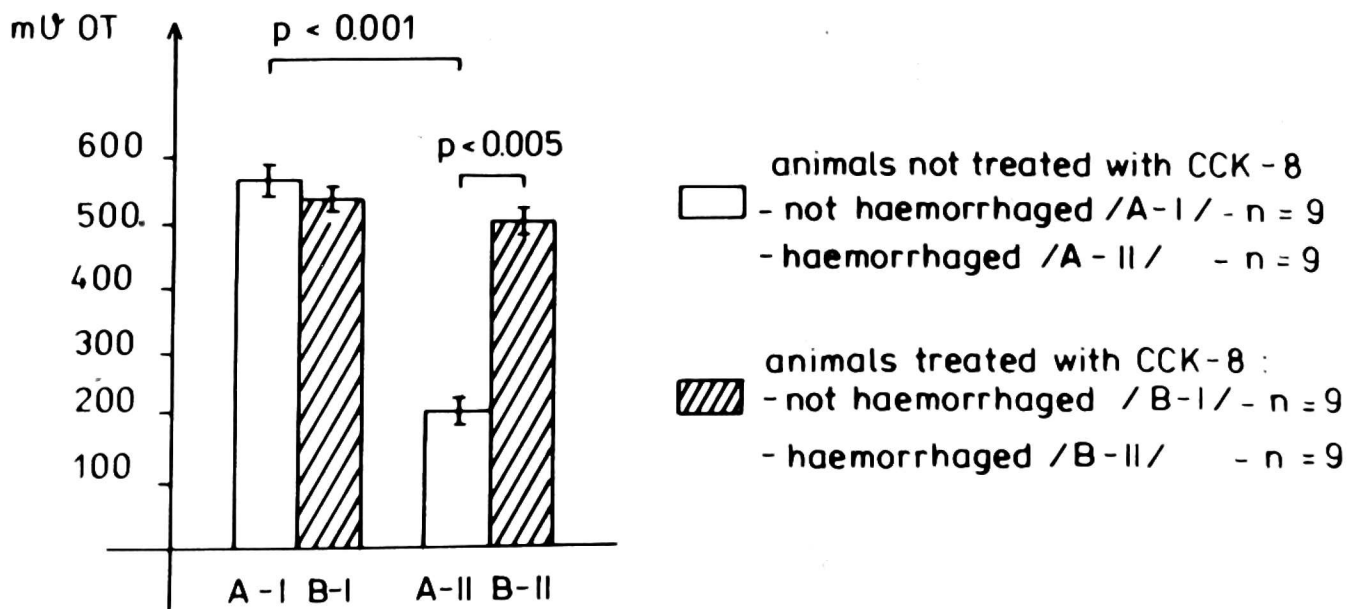

Fig. 2. The neurohypophysial oxytocin content in control and haemorrhaged (1 ml/100 g b. w.) rats following i. c. v. injection of CCK-8 or normal saline (mean ± S. E. M.).
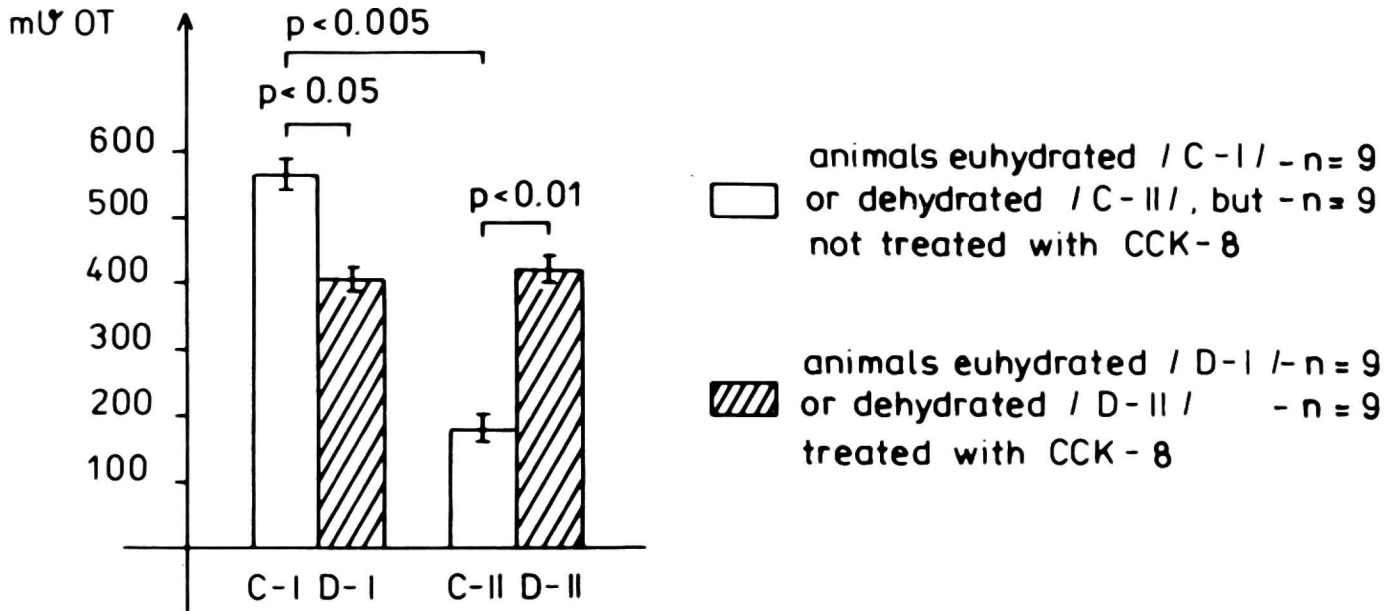
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Fig. 3. The neurohypophysial vasopressin content in control and dehydrated rats following i. c. v. injection of CCK-8 or normal saline (mean ± S. E. M.).
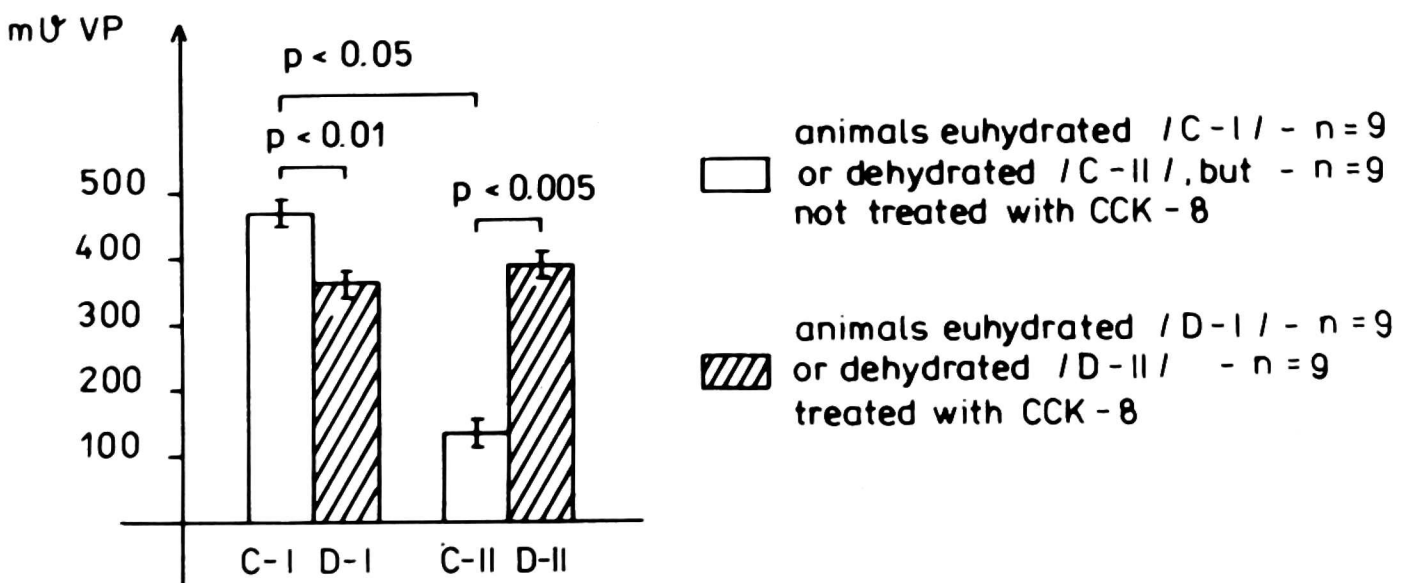

Fig. 4. The neurohypophysial oxytocin content in control and dehydrated rats following i. c. v. injection of CCK-8 or normal saline (mean ± S. E. M.).

DISCUSSION

Usefulness of vasopressin and oxytocin bioassay as compared to radioimmunoassay (RIA).

For a number of years, RIA has been most frequently used for the estimation of vasopressin and oxytocin. RIA is considered to be the method of choice within the field and is thought to have everything to recommend it. However, the immunoreactivity of the neurohypophysial hormones does not perfectly coincide with their bioactivity. The respective blame seems to lie on both sides: on one hand, a higher variability, brought into being on several grounds, is quite usual for bioassay; on the other, some hormonal fragments deprived of biological efficiency may still keep the immunoreactive properties. In this laboratory (22) the correlation coefficient between vasopressin RIA and the pressor bioassay was recently found to be $r = 0.73$; the correlation coefficient between oxytocin RIA and estimation of oxytocin by milk-ejection in vitro was noted to be $r = 0.67$.

The neurohypophysial vasopressin and oxytocin content in dehydrated and haemorrhaged rats.

Dehydration and haemorrhage are both known to be potent stimuli for the release of neurohypophysial hormones. In dehydrated animals the synthesis, infundibular transport as well as the release of vasopressin and oxytocin are increased; the content of both neurohypophysial hormones in the neural lobe progressively dimenishes under conditions of water restriction, the synthesis and axonal transport — although increased — being too poor for compensation of the losses due to increased release into the blood (23). In this respect, the present results are consistent with earlier findings, i. e. with the depletion of both vasopressin and oxytocin in the neurohypophysis of dehydrated animals (24).

Similarly, haemorrhage is known to be followed by an increased release of neurohypophysial hormones. However, the reflex mechanisms involved in an increased release of vasopressin and oxytocin are not identical for dehydration and haemorrhage. Deprivation of water is followed by an increased osmolality of body fluids as well as by secondary hypovolemic hypotension. The mechanism of osmotic control involves, in the rat, osmoreceptors within the anteroventral third ventricle region (AV3V) which contains the organum vasculosum laminae terminalis (OVLT) and the nucleus medianus. There is possibly also a sodium-sensitive receptor mechanism localized in the periven-

tricular region (25). As just mentioned, progressive dehydration does result, moreover, in a hypovolemic hypotension which unloads both the baroreceptors in the atria of the heart (low pressure receptors) as well as those in the carotid sinus and aortic arch (high pressure receptors). As for sudden hypovolemia produced by bleeding, the respective mechanisms are thought to be related to left atrial receptors, the sinoaortic ones having probably smaller role (26); the possible activation of the renin-angiotensin system is supposed to be involved in eliciting secretion of neurohypophysial hormones under these conditions as well (27). Nevertheless, it seems that there is no evidence for any primary involvement of the osmoreceptor system in the mechanisms related to the release of neurohypophysial hormones as brought about by haemorrhage.

The simultaneous release of both vasopressin and oxytocin as brought about by bleeding seems to be still discussed (28). The response (firing pattern) of vasopressinergic neurons to peripheral baroreceptor stimulation is thought to be dissimilar to that of the oxytocinergic ones — cosequently, it is even considered to be an useful index for the electrophysiologic identification of the neurones in question (29). In present experiments, however, we noted a marked decrease of both vasopressin and oxytocin in the neurohypophysis following haemorrhage in the rat. This seems to be consistent with some previous data (30).

The influence of CCK on the vasopressinergic and oxytocinergic neurones.

In recent years a number of papers appeared on the possible relation of oxytocinergic neurones to the feeding behaviour and gastric function as well as on the presumable involvement of CCK in these events. Systemic treatment with CCK-8 has been shown to increase the oxytocin level (but not that of vasopressin) in blood plasma of the rat (29). The antidromically identified oxytocinergic — but not vasopressinergic — neurones were found to be activated following systemic administration of CCK as well as during gastric distension (29). The effects of systemic treatment with CCK on the oxytocin release as well as the inhibition of food intake induced by CCK have been noted to attenuate by gastric vagotomy (31). It may be interjected that ablation of paraventricular nucleus results in hyperphagia and obesity in rats; a crucial role for oxytocin is therefore suggested in a neuronal system related to paraventricular nucleus and mediating neuroendocrine (release of oxytocin), autonomic (gastric motility) and behavioural (feeding inhibition) events, all related to CCK. The reflex mechanism in question seems to be of peripheral origin (29), although — on the other hand — a stimulation of some central nervous mechanisms involving hypothalamic paraventricular neurones following peripheral administration of CCK is admitted as well (32).

The present study confirms previous finding from this laboratory (9) that i. c. v. CCK, when administered over several days to euhydrated animals, diminishes the neurohypophysial stores of vasopressin and oxytocin. It is also consistent with some earlier data (16) that an i. c. v. injection of CCK-8s (synthetic sulfated CCK-8) is followed by increased blood plasma vasopressin in rats.

In present study (dehydration experiments) the possible direct influence of i. c. v. CCK-8 on some links in the afferent nervous chains converging on vasopressinergic and oxytocinergic neurones should be taken into consideration. According to previous suggestion (9) an indirect course of events, related to suppressed thirst, cannot be excluded. It seems hardly possible, however, that the processes which affect vasopressin and oxytocin release in animals dehydrated or haemorrhaged are localized in the neurohypophysis: in in vitro experiments no changes in vasopressin and oxytocin release from the neurointermediate lobes were noted following addition of CCK to the incubation fluid (16).

The present study shows that in animals dehydrated and treated i. c. v. with CCK-8 the depletion of neurohypophysial vasopressin and oxytocin is less marked. Similarly, CCK-8 inhibited the depletion of neurohypophysial vasopressin and oxytocin due to haemorrhage. When CCK-8 was administered prior to bleeding, the neurohypophysial vasopressin and oxytocin contents were not decreased, as it usually happens in rats bled without such pretreatment. What is more, the actual neurohypophysial vasopressin content was at that time even higher than that found in intact animals. Thus, it may be suggested that, under conditions of decreased discharge of left atrial receptors, CCK-8 was possibly able to decrease the vasopressin release down to a level even lower than the amounts liberated into the circulation under basic conditions of euhydration. It may be therefore supposed that afferent impulses of osmoreceptor and/or baroreceptor origin may modify the response of vasopressinergic and oxytocinergic neurones to intracerebroventicular treatment with CCK-8.

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