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Evidence for the Release of Vasopressin and Oxytocin into Cerebrospinal Fluid: Measurements in Plasma and CSF of Intact and Hypophysectomized Rats[1]

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Abstract. Arginine-8-vasopressin (AVP) and oxytocin (OXT) were measured by radioimmunoassay (RIA) in cerebrospinal fluid (CSF) and plasma of Wistar rats under various conditions. In addition, basal plasma levels of OXT were measured in Brattleboro rats, either homozygous or heterozygous for diabetes insipidus (Ho-DI or He-DI respectively). The basal OXT plasma level of Ho-DI rats was elevated as compared to He-DI and Wistar rats. Water deprivation caused a gradual increase of plasma OXT levels in Wistar rats. CSF was collected from Wistar rats by withdrawal from the cisterna magna under pentobarbitone anesthesia. Anesthesia induced a rise of the plasma AVP level as well as of the plasma OXT level. Withdrawal of CSF caused a dramatic rise of plasma AVP levels and, to a minor extent, of plasma OXT levels. In rats studied at 4 weeks after hypophysectomy plasma AVP levels were only detectable after CSF withdrawal. CSF levels of AVP were elevated in these animals.

In intact and hypophysectomized rats, basal OXT plasma levels and the increased levels after anesthesia were the same. The OXT release into the blood was partly impaired after hypophysectomy because CSF withdrawal did not result in a further rise of plasma OXT levels. CSF oxytocin levels were the same in intact and hypophysectomized rats.

A difference of the effect of hypophysectomy was thus observed with respect to the presence in CSF and plasma for AVP but not for OXT. It is concluded that the regeneration of AVP and OXT fibers is different which leads to a different release of the hormones into CSF and plasma.

Hormones of hypothalamic origin are present in cerebrospinal fluid (CSF) under physiological conditions. This includes releasing hormones like thyro-

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tropin releasing hormone [KNIGGE and JOSEPH, 1974] and gonadotropin releasing hormone [JOSEPH *et al.*, 1975] and the neurohypophysial hormones arginine-8-vasopressin (AVP) [HELLER *et al.*, 1968; VORHERR *et al.*, 1968] and oxytocin (OXT) [UNGER *et al.*, 1974]. The presence of AVP and OXT in the CSF is of interest with regard to their effects on the brain [UNGER and SCHWARZBERG, 1970; URBAN and DE WIED, 1975; REES *et al.*, 1976], particularly of AVP since this hormone has been reported to have a role in memory processes in the rat [DE WIED *et al.*, 1975; VAN WIMERSMA GREIDANUS *et al.*, 1975a; VAN WIMERSMA GREIDANUS and DE WIED, in press]. A previous study [DOGTEROM *et al.*, in press] confirmed the presence of AVP in CSF of several species, but did not permit drawing conclusions about the route by which AVP reaches the CSF, because plasma AVP levels of rats were highly elevated as a result of the withdrawal of CSF. Therefore, in the present study AVP and OXT were measured in the CSF of intact and hypophysectomized rats to determine the concentration of the hormones in rat CSF and to obtain information about the route by which they may reach the CSF. The hormone levels were measured in CSF and corresponding plasma samples using radioimmunoassays (RIA). The validity of the OXT-RIA was evaluated by measuring plasma OXT levels in Wistar rats during water deprivation and in plasma of Brattleboro rats, either heterozygous or homozygous for diabetes insipidus (He-DI or Ho-DI).

Materials and Methods

Radioimmunoassays. The RIA of AVP has been described [DOGTEROM, 1977]. The iodination of AVP was performed and the standard curve of antiserum, 'E April', was determined with an extremely potent synthetic AVP preparation (Organon, Oss, The Netherlands, pressor activity in the rat: 509 IU/mg). The sensitivity of the standard curve is 0.25 pg AVP/tube. The cross reactivity with vasotocin is approximately 0.25% and with OXT $< 0.1\%$. AVP was extracted from plasma and CSF samples with activated Vycor glass powder. The recovery was $69.5 \pm 6.5\%$ ($n = 167$) for a range of AVP concentrations (2–32 pg AVP/sample). Plasma of Ho-DI rats was used to measure the recovery of added amounts of AVP and to control for nonspecific effects.

The RIA of OXT was, with minor modifications, the same as the RIA of AVP. The antiserum was raised in rabbits [SWAAB and POOL, 1975]. A synthetic OXT preparation (Organon, depressor activity in the rooster: 450 IU/mg) was used for iodination and standard curves. The iodinated hormone (tracer) was purified twice with a Sephadex G 25 fine column (35×1 cm). The incubation was performed in veronal buffer, pH 9.0. The binding capacity of the OXT antiserum '0-2-Q' with an aliquot of tracer was determined with antibody dilution curves. Standard curves were prepared in triplicate and included 3 tubes with 100 μl buffer plus 10 μl tracer to determine non-adsorbable radioactivity

(diluent blank) and 3 tubes with 50 µl diluted antiserum, 50 µl buffer and 10 µl tracer in order to determine the initial binding (antibody blank = B_0/F_0). B/F values of points of the standard curve which were $\geq B_0/F_0$ were eliminated, indicating the sensitivity of the standard curves [DOGTEROM, 1977]. OXT was extracted from CSF and plasma samples with activated Vycor glass powder. The extraction from plasma was markedly improved by adjusting the pH to 7.0 with 10 µl 2 N HCl per ml. Since no OXT-free plasma is available for control experiments, veronal buffer (pH 7.0), containing albumin (1.25 mg/ml), was used to determine possible nonspecific effects. Additionally, OXT was measured in plasma of Wistar rats at 1 day after hypophysectomy. Recovery was calculated for each experiment by adding standard amounts of OXT to buffer.

Sample collection. Male rats of an inbred Wistar strain (CPB-TNO, Zeist, The Netherlands) weighing 180–200 g were used. The animals were kept 5 per cage and had free access to food and water. The animal house was illuminated from 7 a.m. to 7 p.m. Plasma samples were obtained by decapitation and subsequent centrifugation. In addition, OXT measurements were carried out on plasma of Brattleboro rats (CPB-TNO) (Ho-DI and He-DI). All animals were decapitated between 9 a.m. and 11 a.m. Hypophysectomy was performed under light ether anesthesia via the transauricular route. The animals were studied 4 weeks after hypophysectomy.

CSF samples were collected from intact and hypophysectomized rats. The operation was performed under pentobarbitone anesthesia (6 mg/100 g b.w., i.p.). A hole was drilled into the skull and a PP 25 polyethylene cannula was inserted into the cisterna magna. CSF was withdrawn with a syringe into the PP 25 cannula. The entire procedure took approximately 15 min. During the last 2–3 min 50–100 µl CSF per animal was aspirated. The samples were not or only slightly contaminated with blood. The CSF samples were pooled per group after centrifugation. Blood was collected by decapitation immediately after the operation and plasma was separated. Plasma and CSF was stored at –80°C until tested. Measurements were performed in portions of 200–300 µl of pooled CSF samples and in 1 ml plasma; the extraction volume of CSF samples was adjusted to 1 ml with veronal buffer (pH 7.0).

Calculation and statistics. The data of the RIA measurements were calculated with off-line computer facilities. A log as well as a logit transformation and the best fit linear regression of these values vs pg standard were calculated and plotted. The computer program included correction factors for diluent blank, for sample volume and for the recovery of standards from Ho-DI plasma or buffer. Intra-assay coefficient of variation of the AVP-RIA was 21.1% (assay identical sample containing 16 pg/ml, 18 times in 1 assay). Inter-assay coefficient of variation was 13.9% (assay of sample containing 16 pg/ml in 8 assays in duplicate). The OXT-RIA gave comparable results. Significance of differences was tested with Student's *t*-test. A p value of < 0.05 was considered as significant. All measurements are corrected for recovery.

Results

RIA of OXT

The iodination of OXT produced a tracer with a specific activity varying from 250 to 500 µCi/µg. The elution pattern showed 4 peaks and was

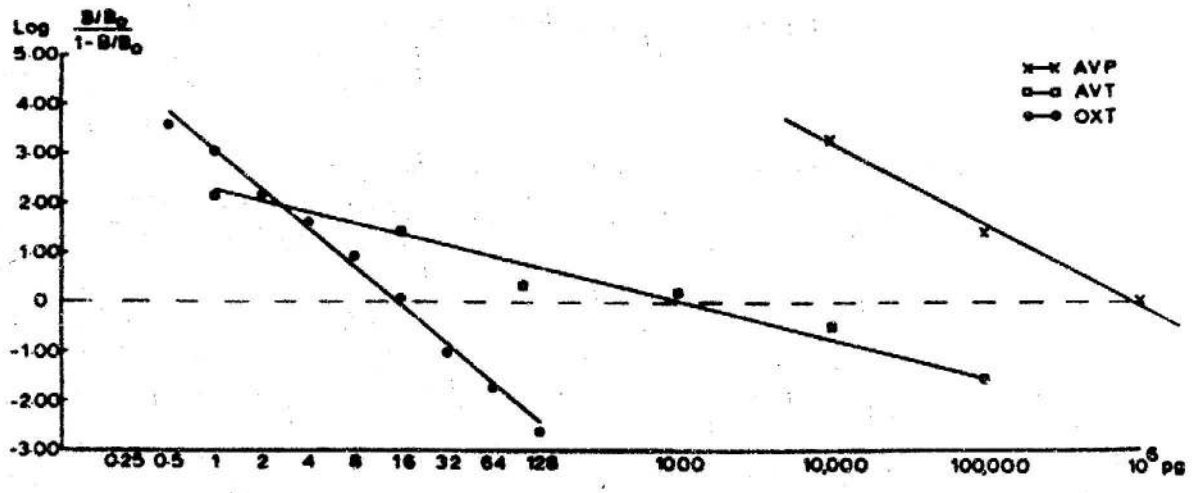

Fig. 1. Representative standard curve of antiserum 0-2-Q in a dilution of 1:8000 (○——○) with OXT as plotted after calculation by computer using a logit transformation. The cross reaction curves with AVP (x——x) and vasotocin (AVT) (□——□) are shown as well.

comparable to the ^{125}I-AVP elution pattern [DOGTEROM, 1977], though the 3rd and 4th peaks appeared after approximately 35 ml and 50 ml eluate, respectively. The maximal binding capacity of 100 μl antiserum 0-2-Q (dilution 1:200) with an aliquot of tracer was 85 to 90% after 2 purifications. Figure 1 shows the standard curve (sensitivity 0.5 pg/tube) and the cross reaction curves with vasotocin and AVP. The cross reactivity with vasotocin is considerable, especially in the lower range of the standard curve, the cross reactivity with AVP being negligible. The extraction procedure resulted in a recovery of 55.8±6.9% (n = 30). The sensitivity of the assay was therefore approximately 2 pg OXT/sample. No nonspecific effects were found in veronal buffer containing albumin.

Measurements of CSF and Plasma

Water deprivation caused a rise of OXT plasma levels in Wistar rats (table I). Considerable variability occurred in the groups with elevated levels. Basal OXT plasma levels in the He-DI rats appeared to be the same as in Wistar rats, whereas basal levels in Ho-DI rats were elevated.

Table II shows AVP and OXT levels in plasma and CSF samples from

Table I. OXT levels in plasma of Wistar and Brattleboro rats

	Plasma OXT levels in pg/ml[1]
Wistar rats	
Basal level	9.4± 2.6 (n = 19)
After water deprivation during 24 h	20.7± 3.6 (n = 15)[2]
48 h	36.2±21.4 (n = 15)
96 h	72.9±30.4 (n = 20)[3]
Brattleboro rats	
He-DI basal level	9.8± 1.8 (n = 8)
Ho-DI basal level	50.5±15.6 (n = 8)[4]

[1] Mean ± SEM; n = number of samples.
[2] $p < 0.01$, basal vs 24 h water deprivation.
[3] $p < 0.05$, basal vs 96 h water deprivation.
[4] $p < 0.01$, He-DI vs Ho-DI.

Table II. AVP and OXT levels in CSF and plasma of intact and hypophysectomized Wistar rats. Values are given in pg/ml as mean ± SEM; n = number of samples

	AVP		OXT	
	Intact[1]	Hypox (4 weeks)	Intact	Hypox (4 weeks)
Basal plasma levels	1.7±0.5 (n=9)	<0.5 (n=8)	9.4±2.6 (n=19)	15.3± 6.8 (n=6)
Plasma levels at 15 min after injection of pentobarbitone	4.5±2.5[2] (n=5)	<0.5 (n=6)	31.7±9.7[3] (n=8)	29.6± 4.5[7] (n=6)
Plasma levels after withdrawal of CSF	29.5±9.5[4] (n=5)	3.8± 0.6[5] (n=24)	60.8±7.3[4] (n=8)	37.7±12.0[8] (n=10)
CSF levels	11.5±3.9 (n=8)	36.9±11.5[6] (n=8)	73.0±6.1 (n=7)	82.8±16.6 (n=6)

[1] These values are shown for comparison. They were presented earlier by DOGTEROM *et al.* in press.
[2] $p < 0.025$, basal vs anesthesia.
[3] $p < 0.01$, basal vs anesthesia.
[4] $p < 0.05$, anesthesia group vs group from which CSF was withdrawn.
[5] $p < 0.001$, intact vs hypophysectomized.
[6] $p < 0.05$, intact vs hypophysectomized.
[7] $p < 0.05$, basal vs anesthesia.
[8] $p < 0.05$, intact vs hypophysectomized.

intact and hypophysectomized rats. Anesthesia induced a rise of plasma AVP and OXT levels in intact animals. Withdrawal of CSF caused an additional rise.

AVP levels were not detectable in plasma samples of conscious or anesthesized hypophysectomized rats. However, release of AVP occurred in these animals after withdrawal of CSF (table II). CSF levels of AVP were elevated after hypophysectomy.

No dissociation of CSF and plasma values was observed for OXT. The basal plasma OXT level tends to be higher in hypophysectomized than in intact rats, though the difference is not significant. An increase of the plasma levels after anesthesia is observed in the hypophysectomized animals. The release of OXT in hypophysectomized animals appeared to be impaired since the increase in plasma OXT levels in these animals after CSF withdrawal was lower than that in intact animals. CSF oxytocin levels are not affected at 4 weeks after hypophysectomy. The measurements of OXT in plasma of Wistar rats at 1 day after hypophysectomy revealed a level of 3.6 ± 1.8 pg/ml ($n = 5$).

Discussion

A RIA of OXT was developed, based on a similar assay of AVP. The results indicate that the assay is capable of detecting basal plasma levels of OXT and that alterations in OXT levels can be measured adequately. No OXT-free plasma was available to control for nonspecific values. However, no nonspecific effects were found in albumin-containing buffer. Secondly, a similar procedure was used for AVP determinations and no nonspecific values were found with this assay in Ho-DI plasma. Finally, the OXT level in plasma of rats at 1 day after hypophysectomy was approximately 30% of the value in intact animals. From these findings we conclude that the values measured are maximally 3 to 4 pg too high. Measurements of plasma OXT levels have been performed by many investigators in pregnant and non-pregnant women, goats, cows, etc., but no reports exist on OXT levels in plasma of male rats. A comparison of our data with the literature data is therefore difficult to make. Preliminary measurements of OXT levels in umbilical cord plasma and in plasma of pregnant women at term [Dogterom, 1977] gave results that were in good agreement with data from literature [Chard *et al.*, 1970; Piron-Bossuyt *et al.*, 1976].

The cross reactivity with vasotocin is high in the low dose range of the

standard curve. This is important because vasotocin-like activity has been demonstrated by bioassay in CSF of anesthetized man [PAVEL, 1970] and of anesthetized cats after intracerebroventricular injection of melatonin [PAVEL, 1973]. Recent measurements in our institute of vasotocin in CSF of Wistar rats with a vasotocin-RIA using a further similar procedure as applied for AVP and OXT measurements, gave vasotocin levels in CSF which were below detection (<7 pg/ml), indicating that no cross reaction has occurred in the measurements of OXT.

The gradual increase of plasma OXT levels after water deprivation is comparable to the rise of AVP [DOGTEROM, 1977] and is in good agreement with an increased activity of the hormone-producing cells in the hypothalamus [SWAAB, 1970] and with the decreasing AVP and OXT content of the posterior lobe [JONES and PICKERING, 1969] during water deprivation. The decreased OXT content of the posterior lobe [VALTIN *et al.*, 1965; VAN WIMERSMA GREIDANUS and DE WIED, in press] in Ho-DI rats is also due to an increased release since increased plasma OXT levels and an increased synthesis in the hypothalamic neurons [SWAAB *et al.*, 1973] were found in these animals.

The present study confirms the presence of AVP and OXT in CSF. The concentrations are comparable to those reported for AVP [VORHERR *et al.*, 1968] and OXT [UNGER *et al.*, 1974]. The CSF level of AVP is increased after hypophysectomy, while the release into plasma is seriously impaired, even after a severe stimulus like withdrawal of CSF. Hypophysectomy eliminates the possibility of transport of hormones via the peripheral circulation and subsequent leakage or release into the 3rd ventricle, the more since an effective blood brain barrier has been reported [VORHERR *et al.*, 1968]. Our data suggest thus a direct release of AVP into the CSF of hypophysectomized rats, either continuously or caused by stress of the withdrawal of CSF under anesthesia. This direct release may be caused by regenerated fibers, located near the 3rd ventricle. On the other hand, AVP might be released into the CSF of intact animals as well, and the elevated values of CSF of hypophysectomized animals could thus be the result of an increased compensatory activity of the hypothalamic synthetizing nuclei after hypophysectomy. Whether in intact animals a direct release of AVP into the CSF exists indeed has to be investigated further in animals bearing permanent cannulas from which CSF and plasma can be sampled without using anesthesia. Immunolocalization of AVP fibers in the median eminence and in the walls of the ventricular system of intact and hypophysectomized rats has to provide additional information.

The high CSF OXT levels suggest that a release of this hormone into CSF, avoiding the peripheral circulation, is also possible. An effective blood brain barrier for OXT has been reported as well [UNGER *et al.*, 1974]. The presence of OXT in plasma of 4 weeks hypophysectomized rats can be explained by regeneration of the neural lobe after hypophysectomy [MOLL and DE WIED, 1962; DANIEL and PRICHARD, 1975]. The normal plasma levels of OXT suggest that regeneration is more effective for OXT neurones as for AVP neurones. These findings are in good agreement with the observations that the milk ejection response is restored at 10 days after hypophysectomy [BINTARNINGSIH *et al.*, 1958], and that water balance is still disturbed 3 weeks after the operation [DE WIED, 1969]. Since hormone release from the external layer of the median eminence has been suggested by OOTA *et al.* [1974] and SILVERMAN and ZIMMERMAN [1975], it is possible that OXT fibers reorganize in such a way that they release earlier during regeneration and more effectively into the portal vessels of this neurohumoral contact zone.

In conclusion, AVP might exert its role in memory processes in the rat via a direct release into the CSF and subsequent transport to the brain regions involved, e.g. to the limbic system [VAN WIMERSMA GREIDANUS *et al.*, 1975b]. However, the memory deficit of hypophysectomized and posterior lobectomized rats, as has been reported by DE WIED [1965], which could be corrected after administration of lysine vasopressin [BOHUS *et al.*, 1973], is contradictory to the high AVP levels in the CSF. The biological significance of the high AVP and OXT levels in CSF has therefore to be further investigated, the more, since recent reports demonstrated that OXT has effects on memory processes in rats which are antagonistic to AVP effects [DE WIED *et al.*, in press]. In that case it might turn out that AVP and OXT in CSF are of no importance for the retention of acquired behavior of rats. The findings of BROWNFIELD and KOZLOWSKI [1977] that neurohypophysin-containing fibers proceed from the hypothalamus to other brain regions, which are currently confirmed in our laboratory with specific immunostaining of AVP and OXT fibers in the septal area and the stria medularis (BUIJS *et al.*, in preparation), point to the possibility that hypothalamic peptides might also exert their behavioral effect via direct neuronal connections. In addition, it remains possible that hypophysectomy or posterior lobectomy eliminates additional factors which are necessary for rats in order to maintain memory consolidation. These may be necessary in order to activate neurogenic peptides or to sensitize the central nervous system for the action of neurogenic peptides.

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