Electric Footshocks Differentially Affect Plasma and Spinal Cord Vasopressin and Oxytocin Levels¹

ANDRÉ F. CRINE* AND RUUD M. BUIJS†

*Hôpital Vincent van Gogh, 55 rue de l'Hôpital, B-6030 Marchienne-au-Pont, Belgium

†Netherlands Institute for Brain Research, Meibergdreef 33
1105 AZ Amsterdam Zuidoost, The Netherlands

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CRINE, A. F. AND R. M. BUIJS. *Electric footshocks differentially affect plasma and spinal cord vasopressin and oxytocin levels.* PEPTIDES 8(2) 243-246, 1987.—Rats exposed for three minutes to repeated electric footshocks showed an approximate 10-fold increase of basal plasma vasopressin (AVP) and oxytocin (OXT) levels. In contrast, spinal AVP and OXT contents measured in the same rats remained unchanged when compared to undisturbed controls. This observation suggests that spinal AVP and OXT do not play a major role in the short-term adaptation of the organism to stress.

Electric footshocks Vasopressin Oxytocin Plasma Spinal cord

THE presence of vasopressin (AVP), oxytocin (OXT) and carrier proteins (Nps) within the spinal cord has been described by a number of researchers using different techniques.

Immunocytochemistry (ICC) [4, 27, 28] has permitted us to visualize these projections throughout the length of the spinal cord including the filum terminale, with preferential innervation of some regions at specific levels (see [28]). OXT stained fibers clearly predominate, AVP fibers appear to be present only in the marginal zone of the substantia gelatinosa and in the region of the central canal [4, 23, 28]. AVP and OXT, as well as Nps, were also detected in the spinal cord by means of radioimmunoassay (RIA) [10, 22, 31]. This procedure confirmed ICC results by showing; first, that the lumbo-sacral part contained the highest amount of both peptides; next, that the peptides were mainly present in the dorsal horn; and lastly, that larger amounts of OXT than AVP were found at all spinal levels. RIA of perfusates from the subarachnoid space of the spinal cord also showed a higher concentration of OXT than AVP [25]. Furthermore, higher concentrations of OXT-Np than AVP-Np have been found in CSF collected at lumbar levels in neurological patients [3].

Although AVP and OXT have been suggested to be implicated in the regulation of various functions (pain [1], temperature [24], sex [2,8], cardiovascular [18] and motor [5,6] activities) that need the spinal cord for transmitting signals, their exact role is not clarified yet (see, for instance, the controversies about the role of intrathecal administration of AVP in regulating pain [21] vs. [30]). It has already been reported that limbic, hypothalamic and brainstem AVP con-

tents may be modified in rats exposed to stressful situations [13-15, 19, 20]. Nothing, however, is known about a possible similar sensitivity of OXT or about the stress response of spinal AVP and OXT contents.

The aim of the present paper is to extend the available information on the sensitivity of cerebral AVP and OXT contents to stress by testing the possibility that the spinal AVP and OXT may be sensitive to a short exposure to intermittent electric footshocks. Plasma AVP and OXT responses have been determined in parallel.

METHOD

Subjects

Twenty male Wistar rats (TNO, Zeist, The Netherlands), weighing 400-450 g at the time of the experiment, were used. They were housed in groups of 4 under a reversed light-dark cycle (lights on from 1.30 p.m. till 1.30 a.m.). Food and tap water were provided ad lib.

Apparatus

It consists of a dark box (40×40×40 cm) equipped with a grid floor (4 mm in dia., spaced 10 mm apart). Scrambled footshocks were delivered through a Grason-Stadler (model 700) shock generator.

Procedure

The animal was put into the dark box and immediately exposed to a series of inescapable footshocks (EFS) (2.5 mA

¹Requests for reprints should be addressed to Dr. P. G. Carlier, Laboratoire de Biochimie, Université de Liège, 17, Place Delcours, B-4020 Liège, Belgium.

TABLE 1
SPINAL AVP AND OXT CONTENTS: LACK OF INFLUENCE OF REPEATED ELECTRIC FOOTSHOCKS

Spinal Cord Segments	n	Treatment	AVP (pg/mg protein)	OXT (pg/mg protein)
Cervical	10	Controls	1.24 ± 0.14*	5.18 ± 0.71
	10	Shocked	1.35 ± 0.21	4.99 ± 0.50
Thoracic	10	Controls	2.27 ± 0.29	10.02 ± 0.94
	10	Shocked	2.17 ± 0.35	9.84 ± 1.03
Lumbar	10	Controls	4.03 ± 0.39	32.51 ± 3.82
	9	Shocked	3.54 ± 0.34	29.17 ± 3.80
Sacro-coccygeal	8	Controls	4.71 ± 0.53	19.48 ± 3.00
	10	Shocked	3.78 ± 0.58	18.95 ± 3.40

*Mean ± S.E.M.

†Mean ± S.E.M. of pooled control and shocked values.

‡Vs. OXT values ($p < 0.002$, two-tailed Student's *t*-test).

§Vs. cervical and thoracic segments ($p < 0.01$, Newman-Keuls test).

*Vs. cervical segment ($p < 0.05$).

#Vs. the 3 other segments ($p < 0.01$).

**Vs. cervical segment ($p < 0.05$).

for 1 sec, every 5 sec) during a 3-min period. It was then transferred to another room and quickly decapitated. The transfer took 30 sec. Control animals ($n=10$) were immediately decapitated after removal from the home cage.

Trunk blood was collected into heparinized plastic test tubes placed on ice and centrifuged at 6,000 rpm for 20 min at 10°C. Plasma was frozen (-20°C) for subsequent RIA. The spinal cord was dissected out and divided into 4 segments: cervical, thoracic, lumbar and sacro-coccygeal. The pieces of tissue were put into plastic test tubes containing HCl 0.1 N, sonicated for 90 sec at 20 kc/sec (MSE 100 Watt Ultrasonic Disintegrator), then frozen (-20°C) until assayed. Protein contents were determined using Lowry's procedure [17]. Two control plasma samples, 1 shocked lumbar segments and 2 control sacro-coccygeal segments were excluded due to technical problems.

Radioimmunoassay for AVP and OXT was carried out as described previously [7]. The AVP and OXT assays had an interassay coefficient of variation of 7.2% ($n=23$) for extracted AVP (8 pg from 1 ml) and of 8.6% ($n=21$) for extracted OXT (32 pg from 1 ml), respectively. The intraassay coefficient of variation for 4 pg AVP was 11.1% ($n=20$) and for 16 pg OXT was 10.2% ($n=20$).

RESULTS

In the case of AVP, the two-way ANOVA revealed a highly significant between-spinal cord segments effect, $F(3,69)=27.0$, $p < 0.001$, but no effect of shock, $F(1,69)=1.26$, N.S., and no shock × spinal level interaction, $F(3,69)=0.7$, N.S. The difference between the spinal cord segments was further analysed using the Newman-Keuls test. As shown in Table 1, the highest and lowest AVP values were found at sacro-coccygeal and cervical levels, respectively. Intermediate values were found in lumbar and thoracic segments, the former displaying higher levels.

TABLE 2
PLASMA AVP AND OXT CONCENTRATIONS

Treatment	n	AVP (pg/ml)	OXT (pg/ml)
Controls	9	1.5 ± 0.3*	4.8 ± 1.0†
Shocked	10	35.0 ± 3.1§	80.6 ± 8.8‡¶

*Mean ± S.E.M.

†Vs. control AVP values ($p < 0.02$, two-tailed Student's *t*-test).

‡Vs. shocked AVP values ($p < 0.002$).

§Vs. control AVP values ($p < 0.002$).

¶Vs. control OXT values ($p < 0.002$).

In the case of OXT, the picture was approximately the same. A large between-spinal cord segments effect was also observed, $F(3,69)=41.4$, $p < 0.001$, but neither the effect of shock nor the effect of shock × spinal level interaction was significant, $F(1,69)=0.7$, N.S., $F(3,69)=0.2$, N.S., respectively. A post-hoc Newman-Keuls comparison indicated that the highest and lowest OXT values were located at lumbar and cervical levels, respectively. Intermediate values were found in the sacro-coccygeal and thoracic parts, the former showing higher values. The contents in lumbar and sacro-coccygeal parts only differed significantly in case of OXT (see Table 1).

The OXT contents were always higher than those of AVP. The ratio was however different according to the level, i.e., 4:1 at cervical, thoracic and sacro-coccygeal levels but 8:1 in the lumbar cord (see Table 1).

In contrast to the absence of shock-induced changes in AVP and OXT spinal contents, AVP and OXT plasma concentrations were strongly increased in response to shocks

(see Table 2). Although the absolute AVP response was significantly lower than that of OXT, $t(18)=4.89$, $p<0.002$ (two-sided Student's t -test), its elevation was 23-fold, $t(17)=10.21$, $p<0.002$, whereas OXT elevation was 17-fold, $t(17)=8.13$, $p<0.002$. The larger AVP response seemed to be mainly due to the significantly lower basal AVP levels than those of OXT, $t(16)=3.16$, $p<0.02$.

DISCUSSION

The results presented here confirm and extend those of Millan and co-workers [22] by showing that AVP and OXT are present in various amounts at various spinal levels. The exposure to inescapable electric footshocks did not however cause any changes in spinal AVP and OXT contents, whereas several modalities of stressor exposure have already been demonstrated, at least as regards AVP, to produce changes in limbic system, hypothalamus and brainstem [13–15, 19, 20].

A number of reasons may be provided to account for both the apparently different shock sensitivity of the cerebral AVP pools and the absence of OXT response. First, it may be that, in our experiment, the time-interval between shock exposure and decapitation was not long enough for spinal changes to occur. Millan and co-workers [19,20], however, have reported increased AVP contents in the medulla-pons of rats subjected to five minutes of foot-shock. Second, it may be that factors controlling peptidergic degradation at spinal levels are different from those of more rostral regions.

The reported increase in peptide content generally observed after a short exposure to stressors is likely to reflect a decreased enzymatic activity and/or an increased local synthesis. These mechanisms might be different in the spinal cord.

Our shock parameters were nevertheless able to augment blood AVP and OXT concentrations, adding EFS to the list of blood OXT releasing stressors [9,16] and confirming their efficacy as blood AVP releasers [11,26]. This peripheral AVP/OXT response to stress may further provide us with a third reason why there are no stress-induced spinal changes. On the one-hand, Knepel and co-workers [12] have recently shown that AVP released after a short exposure to footshock probably originates in the median eminence region. On the other hand, it has been previously reported that separate hypothalamic paraventricular neuronal subpopulations projected to the median eminence and to the spinal cord [29]. In conclusion, it might be tentatively suggested that, in our experimental conditions, paraventricular neurons responsible for the presence of spinal AVP/OXT, contrary to those sending axons to the median eminence, are EFS-unresponsive.

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