Vasopressin and noradrenaline coexistence in the rat locus ceruleus: differential decreases of their levels in distant brain areas after thermal and neurotoxic lesions

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Vasopressin (VP) and noradrenaline (NA) coexist in the rat locus ceruleus (LC). After thermal lesions of the LC, however, no detectable loss of VP-immunoreactive fibers was examined in the brain. VP and NA levels were also measured in the cortex, hippocampus and cerebellum, after unilateral LC lesions. Only significant ipsilateral depletions of NA levels were found. Therefore, no indications were obtained for VP transport by LC neurons towards distant brain areas.

Using immunocytochemistry (ICC), neuropeptides derived from propressophysin, i.e., vasopressin (VP), neuropehyn (NP) and the C-terminal glycopeptide, were demonstrated in many medium-sized multipolar cell bodies of the locus ceruleus (LC) and subceruleus in colchicine-pretreated rats2,3,19,22. Therefore, it was likely that propressophysin is synthesized by LC cells. Subsequently, we found that VP and NP coexist with noradrenaline (NA) in LC cell bodies1, a type of cell which projects throughout the rat neuroaxis12.

The concept of coexistence of monoamines and one or more neuropeptides in neurons is now widely accepted. However, the functional significance of the coexistence of neuroactive substances and their localization at the level of the terminals is generally unclear10. The idea of cotransport of VP and NA to LC terminals was corroborated by biochemical data which show that microinjection of VP in some brain areas modulates local NA turnover23. Furthermore, VP has been reported to potentiate NA-stimulated cAMP formation in mice hippocampi4, which indicates that VP can act on postsynaptic noradrenergic activity and may, consequently, be present in the noradrenergic terminals.

In the present lesion study we investigated the possibility of cotransport of VP in noradrenergic LC neurons towards a number of distant brain areas in which VP fibers of unknown origin are present6.

Adult male Wistar rats, obtained from CPB-TNO (Zeist, The Netherlands), weighing 250–300 g, were used in all experiments. One group received a bilateral LC lesion; survival times: 3 weeks (n = 10) and 6 weeks (n = 10). The same strategy was followed after a unilateral LC lesion. The lesion was produced by radiofrequency using a RFG-4A lesion generator (Inc. Burlington Mass.) with a TCZ/SN 1399 electrode, which has an uninsulated tip with a 0.40 mm diameter. Current was passed through the electrode so that the tip temperature was maintained at 60 °C for 1 min. The coordinates were: −9.3 mm relative to the bregma, 1.0 mm lateral to the sagittal suture and 5.5–6.0 mm below dura, according to the atlas of Paxinos and Watson16. After survival time, the animals were perfused transcardially with 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH

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7.4). Apart from the olfactory bulb and the spinal cord, the brains were removed and postfixed for 3–6 h. Transversal vibratome sections of 70 μm thickness were obtained and subjected to the peroxidase–antiperoxidase procedure (for details see ref. 2), using anti-VP serum (Truus; 9/485, 1:1000).

The VP and NA levels were measured in 3 groups of rats (n = 8). In one group the LC was lesioned unilaterally by radiofrequency, as described above. The second group received a unilateral microinjection of 6 μg 6-OHDA-HCl (Sigma), while a third group received a unilateral microinjection of 20 μg of 6-OHDA-HCl (Sigma) in 0.5 μl saline (containing 0.1% ascorbic acid) into the LC. After a survival time of 21 days the animals were decapitated and 5 brain regions, i.e., the cingulate cortex (CC), frontal cortex (FC), dorsal hippocampus (DH), ventral hippocampus (VH) and cerebellum (CER), were rapidly dissected, subdivided longitudinally into right and left halves, which corresponded to lesioned and non-lesioned sites, and immediately put on dry ice. As soon as the dissection procedure was completed the brain tissue blocks were put in tubes containing 1 ml 0.2 N perchloric acid, homogenized and centrifuged for 10 min at 6000 rpm. The pellet was used to determine sample protein levels and the supernatant was divided into equal aliquots and frozen until use for measurements of VP or NA levels. The site contralateral to the lesion served as an internal control and all values obtained were compared to previously obtained values from non-treated animals (from ref. 24). Brain tissue containing the LC was fixed by immersion in 2.5% glutaraldehyde and Cresyl violet-stained transversal vibratome sections of 50 μm were used to verify the position of the lesion. Samples from the 5 best-lesioned animals, i.e., animals which had the compact part of the LC damaged, were used for measurements.

VP was determined as previously described by Dogterom et al.8. Intra- and interassay variation coefficients were 15.7% and 13.9% respectively at 16 pg VP. The NA levels were assayed according to the radioenzymatic assay used by Van der Gugten et al.20. The sample protein content was determined according to the Lowry method13. The data were expressed as ng/mg protein.

The one-tailed Wilcoxon signed rank test for matched pairs was used to check the hypothesis that the LC lesion causes a significant reduction of VP and NA levels (P ≤ 0.05 was taken as level of significance). A significant reduction is denoted by an asterisk in the tables.

The thermal lesions effectively damaged the compact part of the LC, while local microinjection of 6-OHDA caused paucity of LC neurons. After 3 or 6 weeks of survival time, the brains of both the bilaterally and unilaterally thermal-lesioned animals showed no decrease of VP fibers in examined brain regions when compared to appropriate controls.

After unilateral thermal lesions (Table I) a significant reduction of NA levels was observed in the ipsilateral CC, FC and DH. The NA levels in the VH and CER at the lesioned site of the brain did not show any significant reduction. In this group of animals, VP was not detectable in the CC and CER, either ipsi- or contralateral to the lesion. In the FC, DH and VH the VP levels ipsilateral to the lesion were not reduced significantly compared with the contralaterally lesioned brain site.

The NA and VP values after unilateral microinjec-

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**TABLE 1**

NA and VP levels (in ng/mg protein) after a unilateral thermal LC lesion

The values are given as group mean ± S.E.M. (n = 5).

<table>
<thead>
<tr>
<th></th>
<th>Noradrenaline</th>
<th>Vasopressin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion</td>
<td>Contra</td>
</tr>
<tr>
<td>Cingulate cortex (CC)</td>
<td>0.64 ± 0.20</td>
<td>1.99 ± 0.30*</td>
</tr>
<tr>
<td>Frontal cortex (FC)</td>
<td>0.49 ± 0.10</td>
<td>1.38 ± 0.08*</td>
</tr>
<tr>
<td>Dorsal hippocampus (DH)</td>
<td>1.32 ± 0.11</td>
<td>3.26 ± 0.55*</td>
</tr>
<tr>
<td>Ventral hippocampus (VH)</td>
<td>2.21 ± 0.56</td>
<td>1.86 ± 0.32</td>
</tr>
<tr>
<td>Cerebellum (CER)</td>
<td>1.34 ± 0.44</td>
<td>1.87 ± 0.32</td>
</tr>
</tbody>
</table>

*P < 0.05.
TABLE II

NA and VP levels (in ng/mg protein) after a unilateral microinjection of 6 μg 6-OHDA into the LC

The values are given as group mean ± S.E.M. (n = 5).

<table>
<thead>
<tr>
<th></th>
<th>Noradrenaline</th>
<th></th>
<th>Vasopressin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion</td>
<td>Contra</td>
<td>Lesion</td>
<td>Contra</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>1.48 ± 0.25</td>
<td>2.25 ± 0.19*</td>
<td>&lt;0.08</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>0.68 ± 0.11</td>
<td>1.32 ± 0.26*</td>
<td>0.25 ± 0.06</td>
<td>0.32 ± 0.08</td>
</tr>
<tr>
<td>Dorsal hippocampus</td>
<td>2.33 ± 0.51</td>
<td>2.44 ± 0.26</td>
<td>0.22 ± 0.05</td>
<td>0.27 ± 0.11</td>
</tr>
<tr>
<td>Ventral hippocampus</td>
<td>2.19 ± 0.24</td>
<td>3.09 ± 0.29*</td>
<td>0.34 ± 0.07</td>
<td>0.39 ± 0.08</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.22 ± 0.33</td>
<td>2.65 ± 0.33*</td>
<td>1.22 ± 0.13</td>
<td>1.07 ± 0.10</td>
</tr>
</tbody>
</table>

*P < 0.05.

tion of 6 μg 6-OHDA into the LC are given in Table II. The results indicate that the ipsilateral NA levels were reduced significantly in all selected brain regions except for the DH. In this instance too, the VP values in the CC remained below detection level but again, in the 4 remaining brain areas no unilateral VP effect was observed.

The NA and VP values obtained after unilateral microinjection of 20 μg 6-OHDA are shown in Table III. The results are similar to those obtained in the case of the thermal lesion, i.e., a statistically significant NA reduction was measured in the CC, FC and in the DH but not in the VH and CER. Again, no significant effects were found in the VP levels. It is remarkable that compared with the thermal lesions relatively high VP levels were detected in the CER after neurotoxic lesions.

An important parameter in lesioning experiments is survival time. Ross and Reis18 conducted a study of the activity of dopamine-β-hydroxylase (DBH) in various regions of the rat brain after unilateral thermal LC lesion. The time course and the degree in which DBH activity is depressed indicate LC axonal degeneration and subsequent sprouting. In brain areas similar to those screened by us, they found a significant reduction of DBH activity between the 12th and 40th postoperative day. According to these data our time lapse of 21 and 42 days seems appropriate for detecting axonal degenerative effects of LC lesion. Indeed, the unilateral, thermal and neurotoxic lesions resulted in a unilateral decrease of noradrenaline levels.

The failure to identify VP efferents from the LC after thermal lesions may be explained in at least 3 ways.

Firstly, the ICC procedure applied here may be too insensitive to detect VP in LC efferents as a result of a too low local VP concentration. It is known that the antigen detection ability of the ICC method depends on a complex of factors (e.g. the nature of fixation, avidity of antibodies and local concentration of the antigen)17. Indeed, the sensitivity of the ICC procedure was enhanced considerably in the past decade by modification of the above-mentioned factors.

TABLE III

NA and VP levels (in ng/mg protein) after a unilateral microinjection of 20 μg 6-OHDA into the LC

The values are given as group mean ± S.E.M. (n = 5).

<table>
<thead>
<tr>
<th></th>
<th>Noradrenaline</th>
<th></th>
<th>Vasopressin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion</td>
<td>Contra</td>
<td>Lesion</td>
<td>Contra</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>0.54 ± 0.03</td>
<td>2.06 ± 0.24*</td>
<td>&lt;0.08</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>0.42 ± 0.09</td>
<td>0.79 ± 0.19*</td>
<td>0.68 ± 0.30</td>
<td>0.85 ± 0.40</td>
</tr>
<tr>
<td>Dorsal hippocampus</td>
<td>0.93 ± 0.25</td>
<td>1.95 ± 0.18*</td>
<td>1.16 ± 0.42</td>
<td>0.87 ± 0.28</td>
</tr>
<tr>
<td>Ventral hippocampus</td>
<td>1.90 ± 0.24</td>
<td>2.58 ± 0.38</td>
<td>0.68 ± 0.17</td>
<td>0.70 ± 0.15</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.40 ± 0.37</td>
<td>2.47 ± 0.75</td>
<td>1.16 ± 0.25</td>
<td>1.33 ± 0.26</td>
</tr>
</tbody>
</table>

*P < 0.05.
which resulted in the detection of minute amounts of VP and the elucidation of additional VP systems. Secondly, VP in the LC may not be processed in the same way as the hypothalamic VP systems. Burbach et al. found VP fragments in the rat brain which are not present in the neural lobe. Thus, the various VP systems may process their peptide differentially. Indeed, trypsin pretreatment is necessary to enhance VP immunoreactivity in LC neurons. When LC neurons process VP differentially from, for instance, VP neurons in the hypothalamus, the presently used antisera may be inappropriate for the detection of VP-containing LC efferents. In this case antisera directed against VP fragments may be more appropriate.

Thirdly, VP may not be transported through LC axons to distant brain sites, but (a) is present in a population of LC local circuit neurons or (b) remains in the perikarya. Although it is currently assumed that the majority of VP fibers in the rat LC originates in distant brain regions such as the bed nucleus of the stria terminalis (BST), this does not rule out the possibility that VP neurons in the LC project locally and contralaterally. Indeed it was shown that lesioning of the BST or castration did not result in a complete loss of VP fibers in the LC. In addition, the LC of the rat receives input from the contralateral LC complex (e.g. ref. 11), although this innervation is poor. If all VP-containing LC cells project contralaterally, one would expect more VP fibers. Therefore, the second option is more likely. This idea is in agreement with a report in which it was shown that neuropeptide Y and NA coexist in the LC but do not decline simultaneously in the projection areas after intraventricular injections of 6-OHDA.

The possibility of VP transportation by the LC system to brain regions where VP fibers were not detected by ICC was further examined by RIA measurement of both VP and NA in 5 selected brain areas. The CC and FC were selected because these areas contain the highest cortical NA values, which arise exclusively from the ipsilateral LC. Furthermore, relatively high VP values were measured. The DH was selected because VP is found in this region, microinjection of VP modulates local NA levels and, in addition, VP binding sites occur in this brain part. The VH was chosen because this area contains both VP and NA fibers, VP and NA binding sites, while NA fibers are believed to originate in the LC. The CER was selected because its major noradrenergic innervation stems from the caudal LC — where most cells display both VP and NA immunoreactivity — and, in addition, VP has been demonstrated by RIA. The spinal cord was excluded from analysis by ICC and RIA because VP fibers (originating in the paraventricular nucleus) en passage in the LC region are lesioned as well.

As for the present RIA results, the NA and VP control values did not differ notably from earlier reports. After thermal lesion or injection of 20 µg 6-OHDA, however, the CER and VH did not show a significant NA reduction (mainly due to the small size of the groups) ipsilateral to the LC lesion. The reason is unclear, since, at least in the CER, the DBH activity does decline after a thermal LC lesion. Crossing over of NA fibers may partly explain this phenomenon. However, the significant NA ipsilateral depletion, which is observed in most of the selected brain areas, illustrates the effectiveness of the various LC lesions applied in this study. A remarkable observation was that whereas in the CER the VP levels were below the detection level after thermal lesions, the highest levels (compared with the other structures) were found after neurotoxic lesions. An explanation for this discrepancy is not available at the moment. However, it may be noted that VP immunoreactivity is present in a few Purkinje cells. Under the influence of 6-OHDA these and other cells may be activated in their synthesis of VP.

In conclusion, the present results (ICC and RIA) and those of Gustafson and Moore render it very unlikely that VP and NPY are cotransported with NA through LC axons. The frequently observed event of coexistence of compounds at the level of the perikarya (e.g. ref. 10) does therefore not automatically imply cotransport towards and corelease of these compounds in distant brain areas.

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4 Church, A.C., Vasopressin potentiates the stimulation of cyclic AMP accumulation by norepinephrine, Peptides, 4 (1983) 261–263.


