INHIBITION OF CENTRALLY-EVOKED PRESSOR RESPONSES BY NEUROHYPOPHYSEAL PEPTIDES AND THEIR FRAGMENTS

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Summary—Intracerebroventricular administration of fragments of [arginine[8]]-vasopressin (AVP) such as AVP_{1-6} and AVP_{7-9} attenuated the pressor response evoked by electrical stimulation of the mesencephalic reticular formation in urethane-anaesthetized rats. Oxytocin (OXT) and the fragment OXT_{7-9} were also active, although OXT_{1-6} did not affect the pressor response. These peptides did not influence the bradycardia accompanying the rise in blood pressure, nor the basal blood pressure. The inhibition of the pressor response was shown for OXT_{7-9} to be dose-dependent up to 25 ng. These data suggest that oxytocin, vasopressin and some neuropeptide fragments have an inhibitory role in the regulation of blood pressure. Both the covalent pressinoic ring structure and the C-terminal linear portion of vasopressin contain active sites, while the activity of oxytocin appears to be present in the C-terminal tripeptide Pro-Leu-Gly.

It has become evident that vasopressin influences brain mechanisms involved in adaptive behaviour (De Wied, 1965; De Wied, van Wimersma Greidanus, Bohus, Urban and Gispen, 1976; Kovács, Bohus and Versteeg, 1979) besides the classical peripheral endocrine activities of antidiuresis and vasoconstriction. Vasopressin has also been reported to affect central cardiovascular control. Peripheral (Bohus, 1974) or intracerebroventricular administration of the peptide (Versteeg, Bohus and de Jong, 1979) attenuates increase in the blood pressure (pressor response) and bradycardia evoked by stimulation of the mesencephalic reticular formation of the posterior hypothalamus.

Fragments of vasopressin exert similar behavioural effects to those of the whole molecule (De Wied, 1976; De Wied and Bohus, 1979). Oxytocin, the structurally related neurohypophyseal peptide, exerts behavioural and electrophysiological effects which are the reverse of those seen after administration of vasopressin (Bohus, Urban, van Wimersma Greidanus and De Wied, 1978a). The present experiments were designed to investigate some essential structural requirements necessary for the attenuation of a centrally-evoked pressor response by neurohypophyseal hormones. The pressor response and the bradycardia induced by electrical stimulation of the mesencephalic reticular formation of anaesthetized rats, has been analysed following intracerebroventricular administration of [arginine[8]]-vasopressin (AVP), oxytocin (OXT) and several of their fragments.

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METHODS

Animals and surgery

Specific pathogen-free male Wistar rats (CPB-TNO, Zeist, The Netherlands) weighing between 190 and 240 g were anaesthetized with urethane (1.3 g/kg i.p.). The left iliac artery was catheterized for direct measurement of blood pressure by means of a Statham P23AC pressure transducer and displayed on a Grass polygraph. Heart rate was measured by feeding the arterial pulse wave into a Narco Biosystems biotachometer—BT1200. Rectal temperature was monitored throughout the experiment with a thermistor connected to a teletherm-ometer (Yellow Springs 46 TUC) and body temperature kept constant at 37°C by application of radiant heat.

Stainless steel bipolar stimulating electrodes, diameter 150 μm, insulated, except for the flat end, were implanted stereotaxically in the mesencephalic reticular formation (AP +1.4; RL 1.5; DV +3.0) according to the coordinates of Albe-Fessard, Stutinsky and Libouban (1966). A guide cannula (outer diameter 600 μm) was implanted in a lateral ventricle (AP +7.3; RL 1.2; DV +7.0) for intracerebroventricular administration of peptides or vehicle.

Electrical stimulation was carried out by means of rectangular biphasic pulses from a Grass S88 stimulator. The current was isolated by two Grass SIU 5 stimulus isolation units and the intensity kept constant with the aid of a Grass CCU unit. The duration of each pulse was 1 msec and the delay between the two phases of the biphasic pulse was also 1 msec. The mesencephalic reticular formation was stimulated for 5 sec at frequencies of 10, 30, 50, 70 and 90 Hz with an

Table 1. Structure of neurohypophyseal peptides and fragments

1	2	3	4	5	6	7	8	9		

Cys-Tyr-Phe-Glu-Asn-Cys- Pro-Arg-Gly-NH_2 AVP_{1-9}

Cys-Tyr-Phe-Glu-Asn-Cys- NH_2 AVP_{1-6} (Pressinamide)

Pro-Arg-Gly-NH_2 AVP_{7-9}

Cys-Tyr-Ile-Glu-Asn-Cys- Pro-Leu-Gly-NH_2 OXT_{1-9}

Cys-Tyr-Ile-Glu-Asn-Cys- NH_2 OXT_{1-6} (Tocinamide)

Pro-Leu-Gly-NH_2 OXT_{7-9}

interstimulation interval of 60 sec. In order to obtain a comparable base-line pressor response, the current intensity ($40-100 \mu A$) which caused an increase in systolic blood pressure of 50 mmHg was determined at a stimulation frequency of 50 Hz. This current intensity was then used for stimulation at all frequencies. The frequencies were varied randomly.

Experimental design

Following the determination of the initial pressor response (50 mmHg increase in systolic pressure at 50 Hz stimulation), a random series of stimulations was carried out at the different frequencies. Subsequently, peptides or vehicle were injected into a lateral cerebral ventricle through a guide cannula with the aid of a microsyringe. The stimulation series were then repeated 20, 40 and 60 min after the injection. In one experiment the stimulations were repeated only 40 or 60 min after intraventricular injection. The maximal increase in systolic pressure and the decrease in heart rate during the 5 sec stimulation period served as the measures for pressor and bradycardiac responses.

Peptides

The peptides were dissolved in a drop of 0.01 N HCl and further diluted (1:1000) with 0.9% NaCl.

Acidified saline was used as vehicle. The dose of the peptides was 25 ng and the injection volume $1 \mu l$. The following peptides were used: AVP_{1-6} (pressinamide); AVP_{7-9} (prolyl-arginyl-glycinamide); oxytocin (OXT_{1-9}); OXT_{1-6} (tocinamide) and OXT_{7-9} (prolyl-leucyl-glycinamide). The structure of the peptides is shown in Table 1. In one experiment a dose–response curve for OXT_{7-9} was determined 20 min after injection.

Histological control

After the completion of the experiments, the animals were killed by decapitation and the brain removed and fixed in 4% formalin solution. One hundred micron slices were prepared and stained with 0.1% thionine. The localization of electrodes and the ventricular guide cannula was verified microscopically.

Data analysis

Changes in the pressor response after the intracerebroventricular (icv) injection of peptides or vehicle were calculated as the difference in the magnitude of the increase in systolic blood pressure at a given stimulation frequency before and after treatment (Δ pressor response in mmHg). Similarly, changes in heart rate were calculated and expressed as Δ heart rate in beats/min (bpm).

Statistical analysis of the data was carried out by means of a two-tailed t-test for paired data and Steels' nonparametric multiple comparison test (De Jonge, 1964).

RESULTS

Basal blood pressure and heart rate

The basal blood pressure and heart rate were determined before each stimulation and before the intraventricular injection. A slight increase in basal systolic blood pressure (from 115 ± 3 to 122 ± 3 mmHg of $P < 0.05$ paired t-test) was observed after the first

Table 2. Stimulation frequency-dependent increase in systolic and diastolic blood pressure and decrease in heart rate following electrical stimulation of the mesencephalic reticular formation in urethane-anaesthetized rats

Stimulation frequency (Hz)	Δ Blood pressure* (mmHg)		Δ Heart rate† (bpm)
	Systolic	Diastolic	
10	$0.7 \pm 0.8‡$	0.6 ± 1.0	0.0
30	16.0 ± 1.4	8.6 ± 1.8	-23.1 ± 3.4
50	$51.3 \pm 3.2§$	21.9 ± 3.7	-58.4 ± 11.4
70	77.6 ± 3.3	25.7 ± 3.4	-86.0 ± 14.5
90	84.0 ± 3.4	26.1 ± 4.0	-101.0 ± 13.9

* Maximal increase in blood pressure.
† Maximal change in heart rate.
‡ Mean ± SE of 7 rats.
§ Stimulation intensity to induce an increase of about 50 mmHg in systolic blood pressure at 50 Hz frequency was first determined for each rat. Subsequently, the same stimulus intensity was used for each frequency.

series of electrical stimulations of the mesencephalic reticular formation. In the vehicle-treated controls the blood pressure slowly decreased over the subsequent 60 min after stimulation (109 ± 4 mmHg, $P < 0.05$). The basal blood pressure of rats which received one of the peptides did not differ from that of controls.

The basal heart rate was also increased (from 379 ± 14 to 411 ± 11 bpm; $P < 0.05$) following the first series of stimulations, but remained constant in the control rats during the rest of the experiments. Intracerebroventricular administration of AVP_{1-6}, AVP_{7-9} and OXT_{7-9} caused a slight, but significant ($P < 0.05$) decrease in basal heart rate 20 min after treatment as compared to controls. The heart rate values were as follows: control 401 ± 14 bpm; AVP_{1-6} 389 ± 13 bpm; AVP_{7-9} 375 ± 13 bpm; OXT_{7-9} 367 ± 15 bpm.

Pressor and heart rate response to stimulation of the mesencephalic reticular formation

Stimulation of the mesencephalic reticular formation resulted in a frequency-dependent increase in systolic and diastolic blood pressure, and a decrease in heart rate. The increase in diastolic pressure was monotonic up to 50 Hz, but no further increase occurred at higher stimulation frequencies (Table 2).

The effect of peptides or vehicle on the pressor and heart rate response induced by repeated brain stimulation are summarized in Table 3. The changes caused by the different treatments were similar at all stimulation frequencies, therefore only the data at 50 Hz are shown. The magnitude of the pressor response decreased slightly upon repeated stimulation at 20, 40 and 60 min in control, vehicle-treated rats. A significant diminution of the pressor response was produced by AVP_{1-6} 40 and 60 min after injection, as well as after injection of OXT_{1-9}. The fragments AVP_{7-9} and OXT_{7-9} were also effective in attenuating the pressor response, and the diminution was apparent as early as 20 min after injection. Injection of OXT_{1-6} failed to alter the magnitude of the pressor response significantly. The effect of OXT_{7-9} and vehicle on the pressor response is shown from the original records in Figure 1.

Injection of peptides had a tendency to reduce the accompanying bradycardia. However, due to the large variability in the magnitude of this response, the difference in comparison to controls only occasionally reached statistically significance (OXT_{1-9}; OXT_{7-9}).

Table 4 shows the influence of AVP_{7-9} on the pressor and heart rate response at 50 Hz stimulation frequency, when the stimulations were performed only 40 or 60 min after intraventricular injection in two different groups of rats. A reduction in the pressor response by AVP_{7-9} also occurred when the stimulation was postponed for 40 min. The magnitude of the pressor response was similar to that after a treatment–stimulation interval of 60 min, although at this time the decrease was not significantly different from controls. The peptide AVP_{7-9} failed to affect the mag-

Table 3. Changes in pressor and heart rate response to repeated stimulation of the mesencephalic reticular formation following intracerebroventricular administration of 25 ng of neurohypophyseal peptide hormone fragments

Treatment	Δ Pressor response (mmHg)	Δ Heart rate response (bpm)	Δ Pressor response (mmHg)	Δ Heart rate response (bpm)	Δ Pressor response (mmHg)	Δ Heart rate response (bpm)
Vehicle	−3.7 ± 3.0	−7.0 ± 3.2	−8.9 ± 1.6	−13.6 ± 3.6	−10.4 ± 1.3	−23.0 ± 5.2
AVP_{1-6}	−8.9 ± 1.9	−21.6 ± 6.3	−18.6 ± 3.0*	−30.3 ± 5.3	−23.7 ± 4.0**	−35.6 ± 5.1
AVP_{7-9}	−16.1 ± 2.2**	−22.0 ± 6.5	−24.0 ± 1.9**	−28.6 ± 9.9	−28.9 ± 1.4**	−35.4 ± 12.4
OXT_{1-9}	−12.0 ± 1.6	−25.3 ± 4.4*	−19.7 ± 1.7**	−24.9 ± 8.6	−22.6 ± 2.7**	−27.6 ± 7.2
OXT_{1-6}	−6.1 ± 0.9	−16.0 ± 4.6	−14.3 ± 2.6	−30.4 ± 7.9	−16.4 ± 2.1	−29.0 ± 9.8
OXT_{7-9}	−19.0 ± 3.2**	−27.6 ± 4.3*	−22.1 ± 3.4**	−37.6 ± 7.3*	−24.4 ± 3.4**	−43.0 ± 9.7
Treatment-stimulation interval	20 min		40 min		60 min	

The data represent the mean ± SEM of differences in the magnitude of the systolic pressor response (Δ pressor response) and of the heart rate response (Δ heart rate response in beats per minute) of 7 rats per group, as compared to the pretreatment values.
* $P < 0.05$ test of Steel.
** $P < 0.01$ test of Steel.

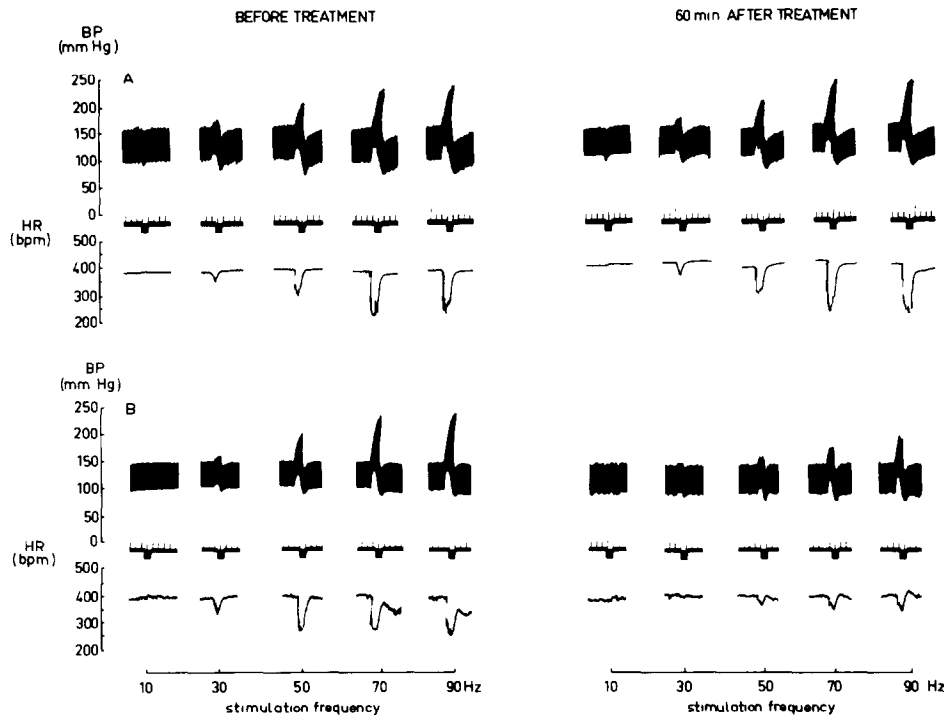BEFORE TREATMENT 60 min AFTER TREATMENT

Fig. 1. Inhibition of the pressor response and bradycardia evoked by electrical stimulation of the mesencephalic reticular formation of an anaesthetized rat by a fragment of oxytocin, 25 ng OXT_{7-9} given intracerebroventricularly (panel B). Recordings of a vehicle-treated control is shown in panel A. For details see Material and Methods.

nitude of the bradycardia which accompanied the pressor response.

Table 5 shows the effects of various doses of OXT_{7-9} on the pressor and heart rate responses 20 min after intracerebroventricular injection. A dose-related inhibition of the pressor, but not the heart rate response, was observed up to a dose of 25 ng at 50 Hz stimulation. No further increase was seen with 50 ng. At the other frequencies the effects were only significant with the 25 and 50 ng doses.

DISCUSSION

The present experiments demonstrate that fragments of vasopressin (AVP_{1-6} and AVP_{7-9}) reduced

the magnitude of a pressor response evoked by electrical stimulation of the mesencephalic reticular formation, in a similar way to the whole molecule (Versteeg et al., 1979, 1982). Since both the covalent ring structure (AVP_{1-6}) and the C-terminal elongation of AVP (AVP_{7-9}) appeared to be effective, it may be concluded that two active sites are present within the vasopressin molecule. The C-terminal tripeptide is somewhat more potent on a weight basis than the covalent ring and the effect of AVP_{7-9} was present 20 min after injection, while AVP_{1-6} was active only after 40 min. The observations with longer treatment-stimulation intervals suggest that the duration of action of AVP_{7-9} was 40 min. Significant effects of the

Table 4. Changes in pressor and heart rate response induced by the stimulation of the mesencephalic reticular formation only 40 or 60 min after intracerebroventricular administration of arginine[8]-vasopressin$_{7-9}$ (AVP_{7-9})

Treatment	Δ Pressor response (mmHg)	Δ Heart rate response (bpm)	Δ Pressor response (mmHg)	Δ Heart reponse (bpm)
Vehicle	-2.9 ± 2.6	-31.6 ± 10.5	-7.7 ± 3.6	-26.4 ± 8.0
AVP_{7-9}	$-16.1 \pm 4.2*$	-28.9 ± 8.2	-14.0 ± 2.7	-17.6 ± 6.5
Treatment-stimulation interval	40 min		60 min	

For details see Table 3. Observations on 7 rats per group.
* $P < 0.01$ (two-tailed t-test).

Table 5. Dose-dependent changes in pressor and heart rate responses induced by the stimulation of mesencephalic reticular formation after intracerebroventricular administration of oxytocin$_{7-9}$

	30 Hz		50 Hz		70 Hz	
	Δ Pressor response (mmHg)	Δ Heart rate response (bpm)	Δ Pressor response (mmHg)	Δ Heart rate response (bpm)	Δ Pressor response (mmHg)	Δ Heart rate response (bpm)
Vehicle	-0.4 ± 1.2	-9.9 ± 3.7	-4.0 ± 1.8	-14.7 ± 7.9	-3.3 ± 2.2	-24.6 ± 7.3
6 ng	-1.0 ± 1.2	-7.3 ± 4.8	-3.9 ± 1.1	-25.9 ± 10.7	-2.7 ± 1.9	-15.3 ± 10.9
12.5 ng	-3.9 ± 1.2	-16.0 ± 4.9	-12.4 ± 2.3*	-25.0 ± 7.6	-11.4 ± 4.3	-33.7 ± 6.5
25 ng	-6.7 ± 1.0**	-4.0 ± 4.5	-23.7 ± 2.5**	-18.4 ± 8.2	-25.7 ± 4.1**	-22.9 ± 12.9
50 ng	-5.7 ± 0.8**	-11.1 ± 6.6	-22.9 ± 2.0*	-24.1 ± 8.4	-27.6 ± 4.5**	-45.1 ± 7.8

For details see Table 3. Observations on 7 rats per dose.
* $P < 0.05$.
** $P < 0.01$.

peptide upon repeated stimulations for 60 min seems to be a carry-over of the former activity rather than a long duration of action.

The existence of two active sites within the vasopressin molecule, one in the pressinoic ring structure and one in the C-terminal linear tripeptide is in agreement with behavioural observations. De Wied (1976) reported that intracerebroventricularly administered AVP$_{1-6}$ (pressinamide) and AVP$_{7-9}$ delayed extinction of an active avoidance response. Retention of passive avoidance behaviour is also facilitated by intraventricularly administered AVP$_{1-6}$ and AVP$_{7-9}$ (Bohus, Kovács, Greven and De Wied, 1978b). Hence different central effects of vasopressin may be mediated through common putative receptor mechanisms.

Oxytocin, the structurally related neurohypophyseal hormone, also causes a reduction in the centrally-evoked pressor response. Behavioural studies have shown that large doses of intracerebroventricularly administered oxytocin may mimick the effects of vasopressin (Bohus et al., 1978a) and that the tocinoic ring structure of oxytocin is very potent in causing vasopressin-like behavioural effects (Bohus et al., 1978b; De Wied and Bohus, 1979). In contrast, smaller doses of oxytocin exert opposite behavioural effects (Bohus et al., 1978a). A decrease in the magnitude of the centrally-induced pressore response is caused by the C-terminal amino acid sequence of the molecule (OXT$_{7-9}$) while the covalent tocinoic ring structure was ineffective. Hence, the active site in the oxytocin molecule appears to be present in the C-terminal elongation. Similar structural requirements have been observed for facilitating the development of morphine tolerance, by both vasopressin and oxytocin. In these studies the tocinoic ring structure appeared to be inactive, while OXT$_{7-9}$ was as potent as the whole molecule (Van Ree and De Wied, 1976, 1977).

The C-terminal portions of the two peptides, AVP$_{7-9}$ and OXT$_{7-9}$, caused comparable attenuation of the centrally-evoked pressor response. The two peptides may act through different mechanisms, although it is also possible that arginine[8] and leucine[8] can be exchanged for each other without loss of activity. Walter, Hoffman, Flexner and Flexner (1975) reported that the C-terminal part of the neurohypophyseal peptide profoundly attenuates puromycin-induced amnesia in mice. Replacement of Leu[8] with Lys, another basic amino acid like Arg, did not eliminate the activity of the tripeptide. Analysis of the dose–response curve of OXT$_{7-9}$ suggests that the effect of this fragment is not a clear monotonic function of the dose. This kind of action of C-terminal fragments seems to be generalized for other biological activities. Flexner, Flexner, Hoffman and Walter (1977) have reported similar dose–response curves for the effect on puromycin-induced amnesia.

Fragments of vasopressin failed to significantly affect the change in heart rate that accompanies the

pressor response. Previous observations suggested
that the pressor response and the bradycardia evoked
by stimulation of the mesencephalic reticular forma-
tion may be controlled by independent mechanisms
(Versteeg *et al.*, 1982). Both responses appear to be
reduced by vasopressin, but Des-glycinamide^9-lysine^8-
vasopressin failed to influence the bradycardia (Ver-
steeg *et al.*, 1979, 1982). Thus, the effect of vasopressin
on the fall in heart rate may be related to the classical
hormonal properties of the peptide. Oxytocin and
OXT$_{7-9}$ had a tendency to reduce the heart rate re-
sponse, but due to large variations the effects did not
always reach statistical significance. That the effects of
these neuropeptides on blood pressure and heart rate
involve separate mechanisms is also supported by the
observations on basal cardiovascular measures. Injec-
tion of AVP$_{1-6}$, AVP$_{7-9}$ or OXT$_{7-9}$ led to a tempor-
ary decrease in basal heart rate without affecting the
blood pressure. Interestingly, these peptides are the
ones that are active in attenuating the stimulation-
induced pressor response. It is not clear yet whether
this correlation is more than a coincidence or not.

The mechanisms by which the action of the pep-
tides on stimulation-induced pressor response is
exerted are not yet eludicated. That these actions were
exhibited by small amounts of peptides injected into a
lateral cerebral ventricle suggests central site(s) for
these effects. The absence of effects on basal blood
pressure suggests that the action of the peptides is
restricted to hypertensive states and unrelated to vas-
cular pressor or depressor activities. However, a
specific action on brain blood flow cannot be ex-
cluded yet.

It has been postulated that neurohypophyseal hor-
mones may serve as precursor molecules for active
peptides with specific functions in the brain (De Wied
and Bohus, 1979). The present study demonstrates
that fragments of vasopressin and oxytocin are active
in attenuating centrally-induced blood pressure re-
sponses, but whether the active fragments represent
physiologically important modulatory principles in
the brain, remains to be elucated. The peptide Pro-
Leu-Gly (OXT$_{7-9}$) may inhibit the release of melano-
tropic stimulating hormone (MSH) from the pituitary
(Celis, Taleisnik and Walter, 1971). Recent studies on
the biotransformation of oxytocin in the rat brain
failed to detect OXT$_{7-9}$ as a biologically occurring
fragment, but other fragments of oxytocin which
might possess various central effects did occur (Bur-
bach, Schotman and De Kloet, 1980).

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