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Barrel Rotation Induced by Vasopressin and Related Peptides in Rats

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KRUSE, H., Tj. B. VAN WIMERSMA GREIDANUS AND D. DE WIED. *Barrel rotation induced by vasopressin and related peptide in rats.* PHARMAC. BIOCHEM. BEHAV. 7(4) 311–313, 1977. — Intraventricular injection of arginine-8-vasopressin and its analogues vasotocin and lysine-8-vasopressin into rat brain evoked a special rotational behavior resembling somatostatin-induced barrel rotation [1]. Oxytocin and oxypressin were less active while vasopressin fragments had no effect. Vasopressin-induced barrel rotation was accompanied by pathological symptoms indicating a disturbance of muscle tone regulation and is considered to be a non-specific and toxic effect. This rotational behavior was not prevented by atropine, propranolol, phentolamine, methysergide or haloperidol but was reduced by chlorpromazine, probably due to the latter's muscle relaxing activity.

Barrel rotation Vasopressin Vasotocin Oxypressin Oxytocin Pressinoic acid Pressinamide Tocinamide Intracerebroventricular injection

IT HAS BEEN reported that high doses of somatostatin injected intracerebroventricularly (i.v.c.) in rats induced an unusual rotational behavior along the sagittal body axis, called barrel rotation [1]. A similar phenomenon observed after i.v.c. injection of arginine-8-vasopressin (AVP) led to investigate this effect of AVP and of some related peptides in more detail and to study the effect of several drugs on peptide induced barrel rotation.

METHOD

Three hundred and eight Wistar male rats (TNO, Zeist) weighing 140–180 g were implanted with plastic cannulas into the right lateral ventricle using the technique described elsewhere [5]. The experiments were performed 1–5 days after the cannulation. Injection volumes were 5 μl/rat administered within 60 sec. The following peptides were used: AVP, lysine-8-vasopressin (LVP), desglycinamide lysine-8-vasopressin (DG–LVP), vasotocin, oxypressin, oxytocin, pressinoic acid, pressinamide, tocinamide and the C-terminal tripeptide of AVP: Pro-Arg-GlyNH$_2$ (Table 1).

Peptides were dissolved in a drop of 0.01 N HCl and further diluted with saline. Peptides were provided by Organon Oss, the Netherlands except somatostatin which was a gift from Dr. R. Guillemin, the Salk Institute, San Diego, U.S.A. The following blocking agents were used: Propranolol (Inderal®, ICI); Phentolamine (Regitine®, Ciba-Geigy); Methysergide (Deseril®, Sandoz); Atropine Sulphate (Merck); Haloperidol (Serenase®, Janssen); Chlorpromazine (Largactil®, Rhône-Poulence). These were dissolved in saline and injected SC (0.5 ml/rat) in general 30 min before peptide administration except for phentolamine and haloperidol which were given 90 min prior to the i.v.c. administration of peptides. For statistics Fisher's Exact Probability Test was used.

TABLE 1

STRUCTURE OF VASOPRESSIN AND RELATED PEPTIDES

1 2 3 4 5 6 7 8 9
Cys-Tyr-X-Gln-Asn-Cys-Pro-Y-GlyNH$_2$

Peptide	X	Y
Arg-8-Vasopressin	Phe	Arg
Arg-8-Vasotocin	Ile	Arg
Oxypressin	Phe	Leu
Oxytocin	Ile	Leu
Lys-8-Vasopressin	Phe	Lys

RESULTS

The results are summarized in Table 2. Typical bouts of barrel rotation (BR) induced by vasopressin and its analogues started immediately or within a few minutes after the i.v.c. injection. Continuous barrel rolling lasted not longer than 2 min but in 5% of the cases alternated with intervals of prostration up to 20 min after the injection. Both clockwise and counterclockwise rotations occurred and the direction occasionally even changed during a bout. About 70% of BR fits were preceded by ataxia, body swaying (lateral head weaving), lying on one side with

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TABLE 2

INDUCTION OF BARREL ROTATION (BR) BY VASOPRESSIN AND RELATED PEPTIDES

Peptide	Dose	BR	Death	approximated ED_{50}*
Vasopressin (AVP)	1.5 ng	0/10	0/10	ca. 200 ng
	8 ng	4/12†	0/12	
	40 ng	4/12†	4/12†	
	200 ng	6/12†	3/12†	
	1 μg	5/10†	1/10	
	5 μg	9/10	5/10†	
Vasotocin	1.5 ng	0/10	0/10	ca. 200 ng
	8 ng	5/14†	1/14	
	40 ng	5/12†	2/12†	
	200 ng	6/12†	5/12†	
	1 μg	3/10†	0/10†	
	5 μg	8/10	6/10†	
Oxypressin	1 μg	0/12†	0/12	ca. 7 μg
	5 μg	5/12†	0/12	
Oxytocin	1 μg	2/13†	0/13	ca. 11 μg
	5 μg	4/11†	0/11	ca. 11 μg
Pressinamide	5 μg	3/12†	0/12	>> 5 μg
	25 μg	0/9		
Pressinoic acid	5 μg	0/12	0/12	>> 5 μg
Tocinamide	5 μg	0/12	0/12	>> 5 μg
Pro-Arg-Gly NH_2	2.5 μg	0/10	0/10	>> 2.5 μg
LVP	0.3 ng	0/11	0/11	ca. 10 ng.
	1.5 ng	4/12†	0/12	
	8 ng	6/12†	1/12	
	40 ng	10/16†	2/16†	
	200 ng	15/18†	4/18†	
DG-LVP	5 μg	0/12	0/12	>> 5 μg

*Dose of peptide inducing barrel rotation in 50% of rats, estimated graphically.
†$p<0.05$ (vs. saline).

spastic limb abduction, body distortions and opisthotonus. These symptoms also could reappear after the bouts of BR, followed by a state of prostration up to a few hours. The animals either died within 5–15 min of treatment due to lung edema, or recovered completely. Rats not displaying BR often showed transient ataxia, head waving and sedation. Other types of behavior observed more frequently after vasopressin than after saline injection were drinking, grooming and "nest building" activity.

AVP, vasotocin and the, in rats, unnatural analogue LVP induced BR over a wide dose range with extremely flat dose-response curves and therefore sometime poor dose-response relationships. The threshold doses were in the 1–10 nanogram range.

Oxytocin and oxypressin also had some BR activity but required higher dose levels (1–5 μg), whereas in the present experiments an amount of 20 μg somatostatin did not evoke any BR. The constituents of AVP and vasotocin tested alone (pressinamide, tocinamide, Pro-Arg-GlyNH_2) had no consistent effect (pressinamide) or were inactive at all in inducing BR, as was DG–LVP. The latter peptide even seemed to have a blocking effect, because after pretreatment with 5 μg DG–LVP i.v.c. LVP no longer produced BR. Since in the saline treated control animals never any BR was observed even a low frequency of peptide induced BR in the experimental groups was already significant ($p<0.05$), according to Fisher's exact probability test. The blocking agents atropine, propranolol, phentol-

amine, methysergide and haloperidol were unable to prevent LVP-induced BR. Only chlorpromazine reduced the incidence and shortened the duration of BR significantly (Table 3).

TABLE 3

INFLUENCE OF SEVERAL BLOCKING AGENTS ON VASOPRESSIN-INDUCED BARREL ROTATION IN RATS

Blocking agent	Dose (mg/kg)	Incidence of barrel rotation
Saline	—	10/11
Atropine	1	7/9
Propranolol	2.5	7/10
Phentolamine	7.5	6/9
Methysergide	5	9/11
Haloperidol	0.5	10/12
Chlorpromazine	5	4/11*

The blocking agents were injected SC 30–90 min prior to 200 ng LVP i.v.c. (see Methods).
*$p<0.05$ vs saline (Fisher's Exact Probability Test).

DISCUSSION

The incidence of barrel rotation after i.v.c. injection of vasopressin and related peptides in rats seems to be a toxic effect, because it was accompanied by other toxic symptoms and frequently led to death (see Table 2). The occurrence of lung edema may indicate also an action on medullar vasomotor and respiratory centers by these peptides. The mode of action of vasopressin in inducing BR is unknown but may not be due to vasoconstriction, because LVP which has less vasoconstrictory activity than AVP was even more potent, while angiotensin II, a more powerful vasoconstrictory agent, failed to cause BR.

Since neither ring nor tail-fragments of AVP were active, both constituents need to be present in the same molecule for the barrel rolling effect. In contrast, the behavioral activity of AVP, i.e., increased resistance to extinction of active avoidance behaviour, is mainly located in pressinamide, the ring of AVP [5]. Replacement of Phe by Ile in the ring (vasotocin) did not alter BR activity, whereas replacement of Arg by Leu in the C-terminal part (oxypressin, oxytocin) led to a remarkable loss of potency.

The phenomenon of barrel rotation itself seems to result from a special type of motor disturbance mainly caused by an (asymmetric) increase in tone of stretch muscles, as indicated by accompanying opisthotonus and spastic distortions. This may be a direct effect of vasopressin not mediated by cholinergic, noradrenergic, dopaminergic or serotonergic functions since none of the blocking agents affected vasopressin-induced BR. The BR-inhibiting effect of chlorpromazine is probably due to its muscle relaxing activity rather than to a dopaminergic blockade, because haloperidol, a more specific dopaminergic blocker without muscle relaxing effect, did not reduce BR. Induction of increased muscle tone is also known from peripheral application of other oligopeptides, e.g., TRH [4]. However TRH injected i.v.c. in rats did not evoke BR, but induced other types of circling behavior and muscle rigidity [2,3]. Similar pathological effects on muscle tone regulation as we observed after vasopressin, e.g., disturbances of balance, gait and motor coordination or spastic rigidity of limb extensors have also been reported for somatostatin [3] and may be responsible for its barrel rotational effect as well. However, there are some differences between the characteristics of BR induced by vasopressin, as observed by us, and somatostatin, as reported by Cohn [1]. These differences are summarized in Table 4.

TABLE 4

DIFFERENCE BETWEEN VASOPRESSIN AND SOMATOSTATIN BY INDUCING BARREL ROTATION IN RATS

Barrel rotation	Vasopressin*	Somatostatin†
Threshold dose	5–10 ng	25 μg
Dose-response relationship	yes	no (?)
Duration of bouts	1–2 min.	ca. 30 min
Lethality	yes	no
Direction of rotations	right or left-handed	left-handed only
Inhibition by atropine	no	yes

*present results.
†According to Cohn and Cohn 1975.

Thus rotational behavior along the body axis can be caused by different peptides and brought about by different modes of action. It may finally be due to pathological peptide effects resulting in disturbance of motor coordination and regulation of muscle tone.

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