Inhibitory effect of atropine on the isoprenaline-induced increase in vasopressin plasma concentration in rats

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Summary. The isoprenaline-induced increase in plasma levels of vasopressin in conscious rats was reduced by intravenous and intracerebroventricular applications of atropine. It is concluded that central neurons with muscarinic receptors contribute to the isoprenaline-induced vasopressin release.

The β-sympathomimetic amine isoprenaline (ISO) increases the release of vasopressin in rats and dogs[1-4]. This effect is probably mediated by the activation of the renin-angiotensin system and the decrease in blood pressure which are induced by ISO[3-5]. The neuronal pathways which report the changes in angiotensin plasma levels and in blood pressure to the hypothalamic nuclei, which regulate the vasopressin release, are unknown. Some of these neurons might form muscarinic synapses, since the stimulation of muscarinic receptors increases the release of vasopressin[6,7]. Therefore, we investigated the effect of antimuscarinic agents on the ISO-induced increase in vasopressin plasma concentration in conscious rats.

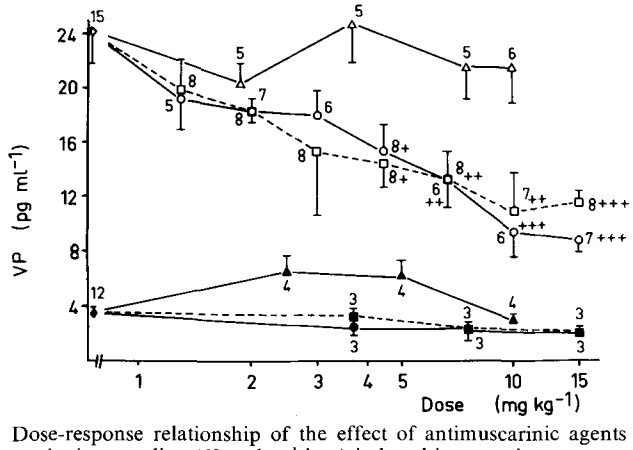
Methods. Male Wistar rats (250-300 g) were used. Blood was collected by decapitation. Column-chromatography with Bio Rex 70 (50-100 mesh; sodium form; pH 5.0) was used for the extraction of vasopressin[4]. Plasma aliquots (3 ml; pH 5.0) were poured on the resin. It was washed twice with 2 ml distilled water and once with 2 ml ethanol (50%; pH 7.0). The resin was eluted with 4 ml ethanol (75%; pH 1.5). The eluates were dried under vacuum. The residues were dissolved in 1.2 ml assay buffer (0.1 M tris (hydroxymethyl)-aminomethane; pH 7.4; 0.02 M disodium EDTA; 0.1% gelatine). 0.9 ml aliquots were used for a radioimmunoassay, which allowed the measurement of 2-800 pg of vasopressin[4]. When 4.7-66.6 pg vasopressin were added to 1 ml plasma, the recovery was 55%. The intra- and interassay variability was 13%. The values which are shown are corrected for recovery.

Plasma renin concentration was determined as has been described previously[8]. Plasma osmolality was measured with a Knauer Osmometer, plasma protein concentration was determined according to Lowry et al.[9]. Mean arterial blood pressure was measured via a cannula (PE 50) in the ventral tail artery, which was connected with a transducer and a Watanabe recorder. Infusions into the left cerebral ventricle were performed under ether anaesthesia. 10 µl of the drug solution of the solvent (0.85% saline) were infused with a Hamilton syringe (2 µl min^{-1}, 1 mm caudal to the coronal and 1.5 mm lateral to the midsagittal suture).

Results and discussion. ISO causes a dose-dependent increase in vasopressin release. The highest vasopressin plasma levels are observed 24 min after the injection of the amine. The ED_{50} is 60 µg/kg^4. I.v. injections of atropine (ATR) and benztropine (BTR), which both pass the blood brain barrier, diminished the ISO-induced vasopressin release (figure). This effect became significant, with doses above 3 mg kg^{-1}. Even 15 mg kg^{-1} of ATR or BTR did not reduce the vasopressin release by more than 50%. Atropine methylnitrate is a quaternary ammonium derivative and does not pass the blood brain barrier. It did not affect the effect of ISO. Thus, the sites of action of ATR and BTR seem to be within the central nervous system.

ATR (10 mg kg^{-1} i.v.) did not change the effect of ISO (60 µg kg^{-1} i.m.) on the factors, which are known to regulate the release of vasopressin. 24 min after the injection of ISO, mean arterial pressure was 84±3 mm Hg (n=7), plasma osmolality 293±1 mOsmol kg^{-1} (n=5), plasma protein concentration 5.9±0.1 g% (n=5) and plasma renin concentration 435±33 ng angiotensin I ml^{-1} h^{-1} (n=13). When ATR was injected 30 min prior to ISO, the respective values were 87±4 mm Hg (n=7), 294±2 mOsmol kg^{-1} (n=5), 6.1±0.3 g% (n=5) and 454±54 ng angiotensin I $ml^{-1}h^{-1}$ (n=5).

ATR was also injected into the left cerebral ventricle (table). The dose which was necessary to reduce significant-



Dose-response relationship of the effect of antimuscarinic agents on the isoprenaline (60 µg kg^{-1} i.m.)-induced increase in vasopressin plasma concentrations (VP pg ml^{-1}). Atropine (□---□) was injected i.v. 30 min prior to isoprenaline; benztropine (○——○) and atropine methylnitrate (△——△) were given i.v. 15 min prior to isoprenaline. In some experiments (filled symbols) the antimuscarinic agents were given prior to the solvent of isoprenaline (distilled water). The antimuscarinic agents were dissolved in 0.9% saline. $\bar{x}\pm$SEM are shown. n, rats/group. Crosses show significant differences to the rats which only received isoprenaline ◇; isoprenaline-solvent ◆. +, $p<0.05$; + +, $p<0.01$; + + +, $p<0.001$.

Intracerebroventricular	Intramuscular	VP (pg ml^{-1})
SOL	-	2.0±0.9 (4)
SOL	ISO 60 µg kg^{-1}	26.9±1.7 (8)
ATR 100 µg	-	1.8±0.5 (4)
ATR 100 µg	ISO 60 µg kg^{-1}	14.0±1.5+ (5)
TRI 100 µg	-	1.6±0.5 (4)
TRI 100 µg	ISO 60 µg kg^{-1}	29.6±3.1 (4)
MEC 100 µg	-	2.5±0.5 (4)
MEC 100 µg	ISO 60 µg kg^{-1}	37.2±2.2+ (4)

Effect of an injection of atropine (ATR), trimethidinium (TRI) and mecamylamine (MEC) into the left cerebral ventricle (i.c.v.) on the isoprenaline (ISO)-induced increase in vasopressin plasma concentration (VP pg ml^{-1}). ISO was injected i.m. ATR, TRI and MEC were injected 30 min prior to ISO. (n). $\bar{x}\pm$SEM. Crosses show significant differences to the respective group of rats, which received an i.c.v. injection of the solvent (SOL) (0.85% saline). +, $p<0.05$.

ly the effect of ISO on vasopressin release, was much smaller than those which had been equieffective after i.v. injection. This action of ATR was not due to a residual effect on nicotinic receptors, which may occur after high doses, since the ganglionic blocking agent trimethidinium (TRI) had no effect on the ISO-induced vasopressin release, whereas mecamylamine (MEC) even slightly increased it.

Based on these data, we conclude that neurons with muscarinic receptors, which are accessible from the ventricular system, contribute to the ISO-induced vasopressin release. It may be speculated that the hypothalamic nuclei, which regulate the release of vasopressin, are informed of the ISO-induced changes in angiotensin plasma levels and blood pressure via different neuronal pathways. Muscarinic synapses may be a part of one of these pathways.

1 G.L. Robertson, R.A. Kinney and A.E. Nelson, Endocrinology *94*, Suppl. 217 A, 164 (1974).
2 W. Knepel and D.K. Meyer, Naunyn-Schmiedebergs Arch. Pharmac. *302*, Suppl. R 44 (1978).
3 D.J. Ramsey, I.A. Reid, L.C. Keil and W.F. Ganong, Endocrinology *103*, 54 (1978).
4 W. Knepel and D.K. Meyer, Naunyn-Schmiedebergs Arch. Pharmac., submitted (1979).
5 T. Berl, P. Cadnapaphornchai, J.A. Harbottle and R.W. Schrier, J. clin. Invest. *53*, 857 (1974).
6 K.P. Bhargava, V.K. Kulshrestha and Y.P. Srivastava, Br. J. Pharmac. *44*, 617 (1972).
7 E.R. Kühn, Neuroendocrinology *16*, 255 (1974).
8 D.K. Meyer and M. Herrmann, Naunyn-Schmiedebergs Arch. Pharmac. *303*, 139 (1978).
9 O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, J. biol. Chem. *193*, 265 (1951).

A study of vasoactive intestinal polypeptide (VIP) stimulated intestinal fluid secretion in rat and its inhibition by indomethacin

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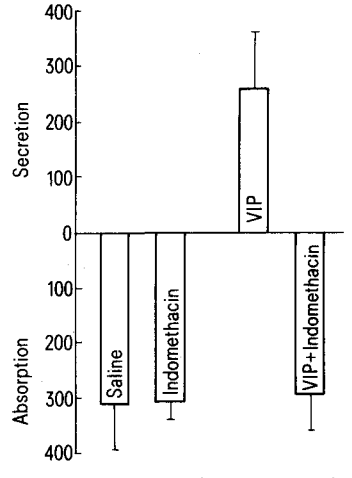
Summary. 4 groups of rats were studied under anaesthesia to assess the effect of VIP and the influence of the prostaglandin synthetase inhibitor indomethacin in isolated bowel loops. VIP produced a highly significant increase in the luminal fluid content and this was completely inhibited by addition of indomethacin.

Porcine VIP is a linear polypeptide with 28 amino acid residues closely related to secretin and glucagon[1]. In humans it occurs at particularly high concentrations in bowel and brain[2] and has been implicated in the watery diarrhoea hypokalaemic achlorhydria (WDHA) syndrome[3]. It is unique[4] among the gut hormones in sharing with prostaglandins[5-7] cholera enterotoxin[8-10] and probably other bacterial toxins, the ability to increase intestinal secretion of fluid[11], activate adenyl cyclase, increase intracellular cAMP in gastric[12] and intestinal mucosa[13] and produce changes in electrolyte secretion. The causal interrelationships and their physiological significance are complex, uncertain and different mechanisms probably operate in each of the above cases - even cAMP itself produces secretory effects resembling prostaglandins[14,15].

Potent inhibitors of prostaglandin synthesis such as aspirin and indomethacin (1-(p-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid)[16-18] produce a short-lived[9] inhibition of enterotoxin-induced secretion. This appeared particularly relevant once it was thought that the normal lag seen during induction of secretion could represent an endogenous process such as prostaglandin synthesis. The present work was undertaken to establish whether VIP increased intestinal secretion in the rat and whether it was inhibited by indomethacin.

Methods. Female Wistar rats (200-250 g) were anaesthetised with 0.1 ml/100 g i.p. pentobarbitone-Na (Sagatal 60 mg/ml) after 18-h fast with unrestricted water. The jugular vein was catheterised (Portex polythene gauge 52) and infused with pentobarbitone (12 mg/40 ml) in half strength physiological saline at 0.03 ml/min. A mid-line abdominal incision followed tracheostomy (Portex polythene PP 205) and the small bowel was tied off with double ligatures into segments approximately 5 cm long from the ligament of Treitz to the caecum. Handling was kept to a minimum and care taken not to occlude the major vessels. Krebs-bicarbonate solution (0.5 ml) was injected without undue distension into each segment using a Gillette 25 G needle. The abdomen was closed and the animal kept warm under a 60 W lamp while pentobarbitone saline with or without added VIP (140 ng/min) was infused for 45 min. Animals receiving indomethacin (Sigma Chemical Co.) were given 1 mg in 0.5 ml anaesthetic fluid over 1 min before the abdomen was opened initially and also 15 min before the infusion of VIP. After 45 min of infusion the abdomen was re-opened and the segments removed. They were first weighed then opened, blotted dry and weighed again. The mean weight gain (+) or loss (−) of the segment, assuming there to be little or no measurable



Intestinal fluid absorption or secretion was recorded as µl/45 min per g wet wt of the sac. There are 5 animals in each group (7 sacs per animal). Bars represent SE of mean.