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INHIBITING THE RABBIT CAUDAL VENTROLATERAL MEDULLA PREVENTS BARORECEPTOR-INITIATED SECRETION OF VASOPRESSIN

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SUMMARY

1. The A1 noradrenergic neurones are known to project from the caudal ventrolateral medulla to the vasopressin-secreting neuroendocrine cells in the hypothalamus. They therefore represent a possible central pathway from the medulla to the hypothalamus for baroreceptor-initiated secretion of vasopressin.

2. We tested this hypothesis in the anaesthetized rabbit. Muscimol, a γ-aminobutyric-acid-receptor agonist, was injected into the caudal ventrolateral medulla to inhibit the A1 noradrenergic neurones.

3. Secretion of vasopressin, measured by radioimmunoassay, was initiated either by arterial haemorrhage or by constriction of the inferior vena cava.

4. After injection of vehicle into the caudal ventrolateral medulla, or after injection of muscimol into nearby control areas, both haemorrhage and constriction of the inferior vena cava produced the expected elevation in plasma vasopressin.

5. After injection of muscimol into the caudal ventrolateral medulla, secretion of vasopressin in response to haemorrhage and to constriction of the inferior vena cava, was completely abolished.

6. The A1 noradrenergic neurones may be the sole pathway transmitting the reflex for baroreceptor-initiated secretion of vasopressin from the medulla to the hypothalamus.

INTRODUCTION

Vasopressin is secreted from the neurohypophysis following haemorrhage or severe hypotension, a reflex originating from baroreceptors within the atria and great vessels (Share, 1974; Wang, Sundet, Hakumaki & Goetz, 1983). Afferent fibres in the IXth and Xth cranial nerves convey baroreceptor information to the nucleus tractus solitarius, whence it reaches vasopressin-secreting neuroendocrine cells in the hypothalamus by an as yet undefined pathway (Clark & Rocha E Silva, 1967; Yamashita & Koizumi, 1979; Kalia & Mesulam, 1980; Spyer, 1982). The caudal ventrolateral medulla contains the A1 noradrenergic neurones (Pl. 1) which project directly to vasopressin-secreting cells in the supraoptic and paraventricular nuclei (McKellar & Loewy, 1981; Sawchenko & Swanson, 1982; Blessing, Jaeger, Ruggiero & Reis, 1982). The nucleus tractus solitarius is known to project to the caudal ventrolateral medulla (Loewy & Burton, 1978; Ricardo & Koh, 1978; Sawchenko & Swanson, 1982), so that

the pathway mediating baroreceptor-initiated secretion of vasopressin may include neurones in this region.

Functional studies support this suggestion. Neurones in the ventrolateral medulla with projections to the hypothalamus are affected by baroreceptor-derived input (Ciriello & Caverson, 1984*a*, *b*). Excitatory agents applied to the caudal area elevate plasma vasopressin (Bisset, Feldberg, Guertzenstein & Rocha E Silva, 1975; Feldberg & Rocha E Silva, 1978; Sved, Blessing & Reis, 1985). Inhibitory agents, applied to the same area, prevent the release of vasopressin normally seen after carotid occlusion (Feldberg & Rocha E Silva, 1981). Although lesioning the A 1 cell bodies in the rabbit increases plasma vasopressin (Blessing, Sved & Reis, 1982), subsequent studies have shown that this is due to an initial excitatory effect of the lesions (Blessing & Willoughby, 1985*a*, *b*). Although some electrophysiological studies show that microionophoretically applied noradrenaline inhibits magnocellular neurones (Barker, Crayton & Nicoll, 1971; Moss, Urban & Cross, 1972; Arnauld, Cirino, Layton & Renaud, 1983) more recent studies have demonstrated a convincing excitatory effect (Day & Renaud, 1984; Day, Ferguson & Renaud, 1984; Tanaka, Kaba, Saito & Seto, 1984; Kannan, Yamashita & Osaka, 1984).

In the present study we have tested the hypothesis that one of the functions of A 1 noradrenergic neurones is to act as an excitatory link in the central pathway mediating the secretion of vasopressin in response to haemorrhage and hypotension. We have done this by measuring the baroreceptor-initiated secretion of vasopressin after inhibiting neuronal function in the caudal ventrolateral medulla using local application of muscimol, a long acting γ-aminobutyric-acid-receptor agonist (Johnston, Curtis, de Groat & Duggan, 1968; DeFeudis, 1980).

METHODS

Animals and surgical procedures

Male New Zealand White rabbits (2–3 kg) were used. They were housed with free access to food and water, and were transferred to the laboratory in small cages in which they remained while experiments without general anaesthesia were performed. Preparatory surgical procedures were carried out under halothane anaesthesia, one to two weeks before experiments. Experimental studies in anaesthetized animals were carried out under urethane (1·4 g/kg), infused over 30 min into a marginal ear vein. After establishment of anaesthesia, the rabbit was intubated and mechanically ventilated with oxygen-enriched air after muscle relaxation with curare (0·75 mg/kg). Rectal temperature was maintained at 38 °C.

In the preliminary operation, in some animals, an inflatable-cuff constrictor was placed around the inferior vena cava, just above the diaphragm (Korner, Shaw, West & Oliver, 1972). Tubing, connected to the cuff, was left subcutaneously in a dorsal position, from which it could subsequently be retrieved. By graded inflation of the cuff it was possible to reduce venous return, thereby lowering atrial filling and systemic arterial pressure.

Experiments on the medulla oblongata were performed under anaesthesia with the head fixed in a Kopf stereotaxic holder. The medulla was exposed by incision and retraction of the atlanto-occipital membrane, without affecting the occipital bone or the cerebellum. The degree of neck flexion was adjusted so that the dorsal surface of the medulla was horizontal. The medullary surface was covered in warm Ringer solution.

Measurement of arterial pressure, heart rate and blood gas tensions

Arterial pressure and heart rate were recorded on a Grass Model 7 Polygraph via a Statham P23 ID pressure transducer connected to a catheter in the central ear artery (unanaesthetized animals)

or in the femoral artery (anaesthetized animals). Mean arterial pressure was obtained by electronically damping the phasic signal and heart rate (heart rate) obtained from the pulse wave with a Grass Model 7P4F tachograph. Arterial blood gases were measured on 1 ml samples using an IL 513 pH/Blood Gas Analyser (Lexington, MA).

Measurement of plasma vasopressin

Blood (1·3 ml) for assay of vasopressin was obtained from the arterial catheter and replaced immediately by warm Ringer solution. Samples were heparinized, stored on ice and centrifuged within 3 hours. The resulting plasma was stored at −20 °C until assay. Concentrations of vasopressin were determined by radioimmunoassay, using an antibody supplied by Dr J. D. Fernstrom, Pittsburg, U.S.A., following extraction from plasma by cation exchange chromatography (Van Itallie & Fernstrom, 1982). Recovery after extraction was 75–100 %. Vasopressin for iodination was purchased from Bachem (California). Bound and free vasopressin were separated using polyethylene glycol. The sensitivity of the assay was 1–128 pg/tube, and the intra- and interassay variabilities were 13 % and 14 % respectively. The assay failed to detect oxytocin at a concentration of 32 pg/tube and crossreacted 2·5 % with 64 pg/tube oxytocin.

Intramedullary injections

Stereotaxic injections were made through long, fine, glass micropipettes with bevelled tips (o.d. 50 μm). The experimental site, where A1 neurones are located (Blessing, Chalmers & Howe, 1978), was 1 mm caudal to the rostral border of the area postrema, 3 mm lateral to the midline and 3 mm below the dorsal surface of the medulla. Control sites (Pl. 1) were either 2·5 mm dorsal or 1·5 mm medial to the experimental site. Muscimol hydrochloride (Sigma), 1 nmol per 0·2 μl of Ringer solution, was injected bilaterally in approximately 2 s. The Ringer solution contained 0·1 % horseradish peroxidase (Sigma) to mark the centre of the injection site. Micropipettes were removed 1 min after injection.

Experimental protocol

Unanaesthetized rabbits – inferior vena cava constriction. A central ear artery cannula was inserted and the subcutaneous tubing for cuff inflation located. After a 15 min rest period, control values for mean arterial pressure, heart rate and plasma vasopressin were obtained. The inferior vena cava cuff was then inflated for 60 or 90 s, using sufficient inferior vena cava constriction to reduce mean arterial pressure by 20–70 mmHg. Plasma vasopressin was measured 1 and 5 min after deflation of the cuff. After results from these pilot experiments were obtained, we performed further experiments in which the inferior vena cava cuff was inflated to a degree sufficient to reduce mean arterial pressure by at least 30 mmHg for 3 min. Arterial blood gases were measured during the last 15 s of the constriction period and mean arterial pressure, heart rate and plasma vasopressin were measured 1 and 5 min after cuff deflation.

Anaesthetized rabbits – inferior vena cava constriction. Control values for mean arterial pressure, heart rate, plasma vasopressin and blood gases were obtained after surgical preparation. Either Ringer solution (0·2 μl) or muscimol (1 nmol in 0·2 μl of Ringer solution) was then injected bilaterally into the caudal ventrolateral medulla. Cardiovascular variables and plasma vasopressin were reassessed after 5 min. The cuff constrictor was then inflated for 3 min. A sample for blood gas assessment was obtained during the final 15 s. Plasma vasopressin was measured 1 and 5 min after cuff deflation.

Anaesthetized rabbits – haemorrhage. Rabbits without inferior vena cava cuffs were used. Control values were obtained for all variables and then either vehicle or muscimol was injected into the caudal ventrolateral medulla. In additional control experiments, muscimol (1 nmol) was injected into either the dorsal or the medial site (Pl. 1). Variables were reassessed after 5 min. Blood was then withdrawn from the femoral artery (15–20 ml/kg) to reduce mean arterial pressure to approximately 40 mmHg. This procedure occupied approximately 3 min. Blood gas analysis was performed on the last blood removed. Plasma vasopressin was measured after a further 2–5 min. The blood was then returned to the rabbit to ensure survival for histological analysis of the injection site.

Histological analysis

Accurate localization of microinjections was confirmed at the conclusion of each experiment by perfusing the animal with fixative and processing the brain for catecholamine fluorescence histochemistry (Blessing *et al.* 1978), horseradish peroxidase activity and for Nissl substance. Some sections, from other rabbits, not injected with peroxidase, were processed for tyrosine hydroxylase immunoreactivity (Pl. 1*C*), using an antibody provided by Dr T. H. Joh, New York, U.S.A. The antiserum was used at a dilution of 1 in 5,000 and the sections processed using the avidin–biotin–peroxidase procedure (Vectastain, CA).

RESULTS

Control levels of plasma vasopressin

Resting plasma vasopressin values were invariably less than 10 ng/l in unanaesthetized rabbits (mean 4 ± 2 ng/l, $n = 23$). Approximately 10% of urethane-anaesthetized rabbits had control levels greater than 20 ng/l. We excluded all data from these animals from further analysis. Elevated resting vasopressin levels were likely to occur if the anaesthetic was infused too rapidly or if transient hypotension occurred during surgical procedures.

Plasma vasopressin after constriction of the inferior vena cava and blood gas analysis

In pilot experiments we varied the duration and degree of constriction of the inferior vena cava, recording the fall in mean arterial pressure and the rise in heart rate during constriction, and measuring plasma vasopressin levels 1 and 5 min after cessation of constriction. The unanaesthetized rabbits rested quietly in their cages during these procedures. Constriction periods of 60, 90 and 180 s all increased plasma vasopressin. The 5 min level was approximately 40% of the 1 min level, in both unanaesthetized rabbits and in anaesthetized rabbits subjected to 180 s of inferior vena cava constriction (Fig. 1). This is consistent with published values of approximately 4 min for the plasma half-life of vasopressin (Lauson, 1974), indicating that vasopressin secretion ceased promptly after restoration of venous return through the inferior vena cava, in both unanaesthetized and anaesthetized rabbits.

For the 90 s constriction period, in unanaesthetized rabbits, we systematically adjusted the degree of balloon inflation to produce graded falls in mean arterial pressure. This resulted in a significant relationship between the fall in mean arterial pressure and the subsequent post-constriction value for plasma vasopressin (Fig. 2), a relationship also present in anaesthetized rabbits after injection of vehicle into the caudal ventrolateral medulla.

Both inferior vena cava constriction and arterial haemorrhage caused a metabolic acidosis, without a major change in pH because a degree of respiratory alkalosis developed. Hypoxaemia was never observed.

These results suggest that the constriction stimulus used resulted in secretion of vasopressin by a baroreceptor-mediated mechanism.

Inferior vena cava constriction in anaesthetized rabbits after intra-medullary injections

Injection of Ringer solution into the caudal ventrolateral medulla did not affect mean arterial pressure, heart rate or plasma vasopressin (Fig. 3). Subsequent constriction of the inferior vena cava for 3 min reduced mean arterial pressure and

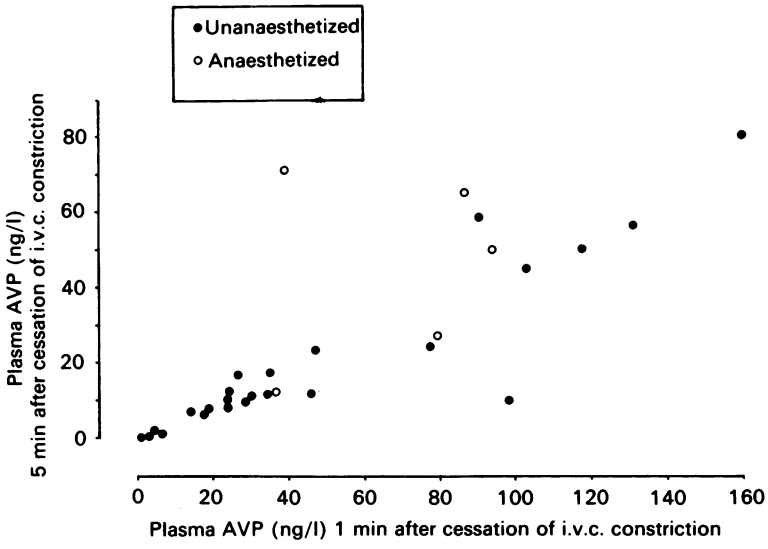

Fig. 1. Relationship between plasma vasopressin (AVP) values obtained 1 and 5 min after ceasing constriction of the inferior vena cava (i.v.c.). Data are from unanaesthetized rabbits (constriction for 60, 90 or 180 s) and from anaesthetized rabbits after injection of vehicle into the caudal ventrolateral medulla (constriction for 180 s). Linear regression was significant ($R = 0{\cdot}91$, $P < 0{\cdot}01$).

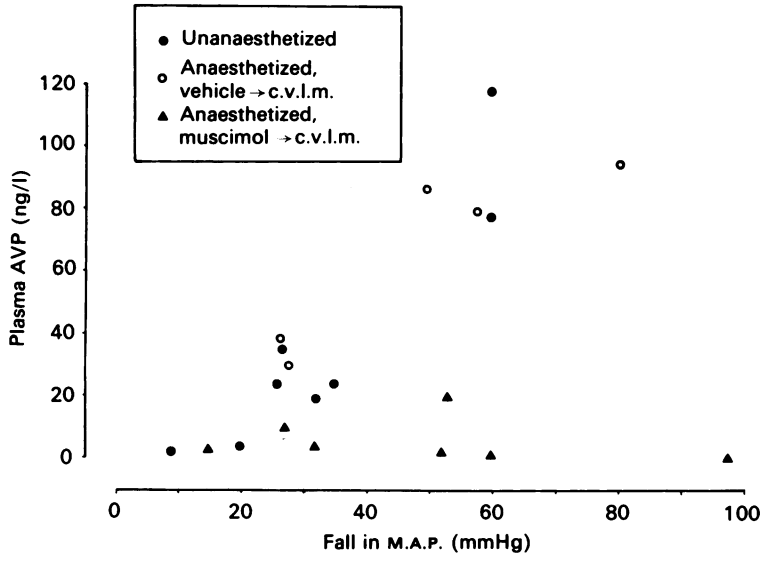

Fig. 2. Relationship between fall in mean arterial pressure (M.A.P.) and plasma vasopressin measured 1 min after ceasing constriction of the inferior vena cava. The inferior vena cava was constricted for 90 s in unanaesthetized rabbits (●) and for 3 min in anaesthetized rabbits after injection of either vehicle (○) or muscimol (▲) into the caudal ventrolateral medulla (c.v.l.m.). Linear regression was significant for both the unanaesthetized rabbits ($R = 0{\cdot}90$, $P < 0{\cdot}01$) and for the anaesthetized rabbits after injection of vehicle ($R = 0{\cdot}92$, $P < 0{\cdot}05$). For muscimol-injected animals there was little or no increase in plasma vasopressin for any fall in mean arterial pressure.

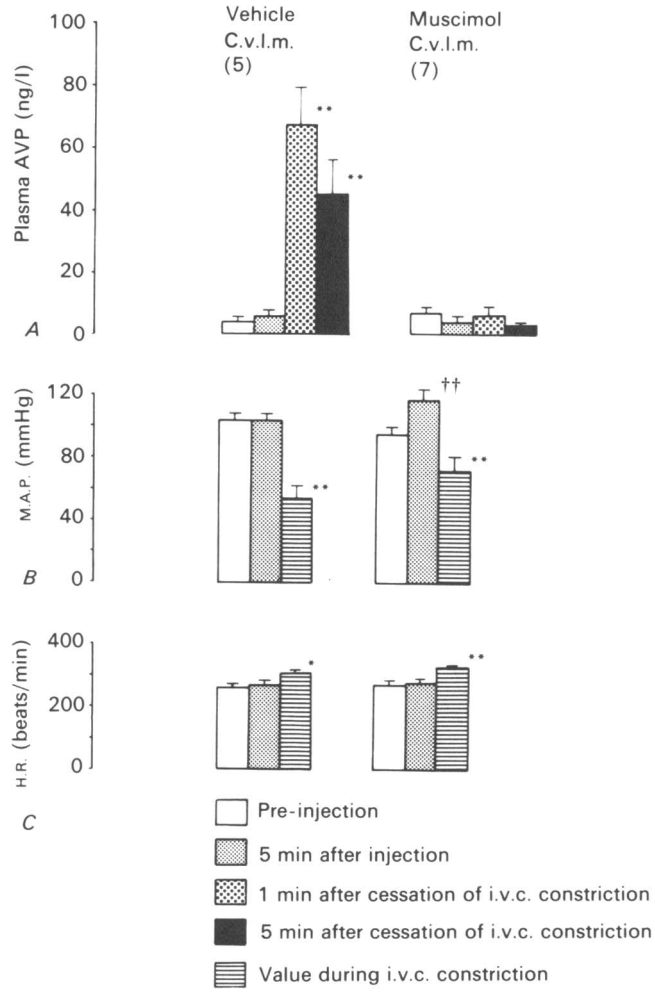

Fig. 3. *A*, effect of injection of muscimol (1 nmol) into the caudal ventrolateral medulla, on secretion of vasopressin secondary to constriction of the inferior vena cava (mean ± S.E. of mean). *B* and *C*, corresponding results for mean arterial pressure (M.A.P.) and heart rate (H.R.). Number of rabbits in each group is shown in parentheses. *, significantly different from the corresponding value immediately before inferior vena cava constriction, $P < 0.05$, paired *t* test. **, significantly different from the corresponding value immediately before inferior vena cava constriction, $P < 0.01$, paired *t* test. ††, significantly different from the corresponding value before injection of muscimol, $P < 0.01$, paired *t* test.

increased heart rate (Fig. 3), as also occurred in the unanaesthetized rabbits. One minute after cessation of inferior vena cava constriction, plasma vasopressin had increased from 6 ± 2 to 67 ± 12 ng/l (Fig. 3). By 5 min this value had decreased to 45 ± 11 ng/l.

Injection of muscimol into the caudal ventrolateral medulla elevated mean arterial pressure without changing plasma vasopressin or heart rate (Fig. 3). Subsequent inferior vena cava constriction caused the expected fall in mean arterial pressure and

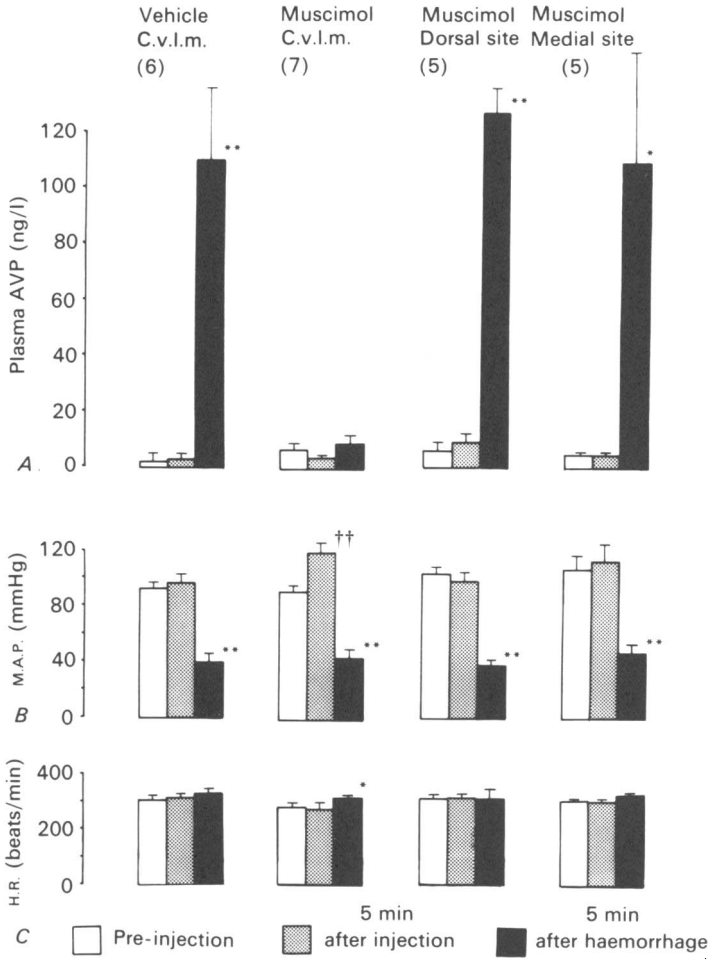

Fig. 4. *A*, effect of muscimol (1 nmol), injected into the caudal ventrolateral medulla or into control sites dorsal and medial to the caudal ventrolateral medulla, on the release of vasopressin secondary to arterial haemorrhage (mean ± S.E. of mean). *B* and *C*, corresponding data for mean arterial pressure (M.A.P.) and heart rate (H.R.). Number of rabbits in each group shown in parentheses. Significantly different from previous corresponding value, $*P < 0.05$, $**P < 0.01$, paired *t* test.

increase in heart rate, but plasma vasopressin remained entirely unchanged from pre-constriction values (Fig. 3). Constriction of the inferior vena cava in vehicle and muscimol injected groups caused similar falls in mean arterial pressure (50 ± 10 and 49 ± 10 mmHg respectively) and rises in heart rate (60 ± 16 and 42 ± 13 beats/min) but the final mean arterial pressure was slightly higher in the muscimol-injected group (72 ± 9 compared to 54 ± 9 mmHg), because mean arterial pressure was higher in this group immediately before inferior vena cava constriction. Maximal inferior vena cava constriction readily reduced arterial pressure to levels as low as 30 mmHg in vehicle-injected rabbits. In muscimol-injected animals it was usually impossible to reduce pressure below 60 mmHg even with maximal inferior vena cava constriction.

However, analysis of results for individual rabbits in the muscimol-injected group (Fig. 2) revealed no relationship between fall in mean arterial pressure and subsequent plasma vasopressin. Indeed the muscimol-injected animal with the most effective hypotensive stimulus (mean arterial pressure falling from 138 to 40 mmHg) had no detectable rise in plasma vasopressin (Fig. 2).

Haemorrhage in anaesthetized rabbits after intra-medullary injections

Injection of Ringer solution into the caudal ventrolateral medulla or injection of muscimol into dorsal and medial control sites did not alter mean arterial pressure, heart rate or plasma vasopressin (Fig. 4). After arterial haemorrhage in these rabbits, plasma vasopressin increased to over 100 ng/l (Fig. 4). Injection of muscimol into the caudal ventrolateral medulla increased mean arterial pressure without change in plasma vasopressin (Fig. 4). Before haemorrhage, plasma vasopressin was 4 ± 1 ng/l. After haemorrhage it was 9 ± 3 ng/l, not significantly changed from the pre-haemorrhage value ($P > 0{\cdot}05$, paired t test). The level of mean arterial pressure after haemorrhage was 44 ± 6 mmHg, not significantly different from the corresponding level in the control group (39 ± 7 mmHg, $P > 0{\cdot}05$, t test for independent means). To reduce mean arterial pressure to basal levels, it was necessary to remove more blood (20 ml/kg) from the muscimol-injected animals than from the vehicle-injected animals (15 ml/kg).

DISCUSSION

The results presented show that injection of muscimol into the caudal ventrolateral medulla prevents the release of vasopressin normally seen after haemorrhage or hypotension. The medullary region where muscimol had this effect was shown to be restricted. Injections dorsal or medial to the caudal ventrolateral medulla did not affect the release of vasopressin. The effective site includes A 1 noradrenergic neurones and, as can be seen in Pl. 1, these cells and their dendritic processes correspond with the injected region.

Inhibition of vasopressin secretion by application of γ-aminobutyric-acid-receptor agonists to the caudal ventrolateral medulla is in accord with increases in the level of this hormone observed after blockade of the same receptors with bicuculline (Feldberg & Rocha E Silva, 1978; Sved, Blessing & Reis, 1985). These two results, taken together, suggest that A 1 neurones stimulate vasopressin-secreting cells and, in turn, are themselves tonically inhibited by a γ-aminobutyric-acid-containing input. The latter may well derive from cell bodies in the nucleus tractus solitarius (Blessing, Oertel & Willoughby, 1984).

We used reasonably physiological methods to produce baroreceptor-initiated secretion of vasopressin. We were careful to maintain arterial oxygen tension, thereby minimizing the chemoreceptor stimulation which would be induced by carotid occlusion, as used by Feldberg & Rocha E Silva (1981). Changes in cerebral blood flow, secondary to haemorrhage and reduced venous return, presumably would be similar in both our control and our experimental groups.

We again observed an increase in arterial pressure following activation of γ-aminobutyric acid receptors in the caudal ventrolateral medulla, in agreement with

previous studies in the rabbit (Blessing & Reis, 1982, 1983) and the rat (Willette, Kreiger, Barcas & Sapru, 1983). This finding differs from that of Feldberg and his collaborators. Although Feldberg & Guertzenstein (1976) described a caudal nicotine-sensitive vasodepressor area on the ventral surface of the medulla of the cat, no increase in arterial pressure was detected when γ-aminobutyric acid was applied to this region (Feldberg & Rocha E Silva, 1981), nor did bicuculline cause dramatic hypotension (Feldberg & Rocha E Silva, 1978), as occurs in the rabbit and the rat (Blessing & Reis, 1983; Willette, Barcas, Kreiger & Sapru, 1984). Recent work in the cat, using glutamate-induced excitation, has re-emphasized the importance of the caudal vasodepressor area (McAllen & Woollard, 1983). Guertzenstein & Lopes (1984) have shown that inhibition of the caudal region with pentobarbitone elevates arterial pressure and it is likely that neuroactive amino acids will also prove to have reciprocal effects on vasomotor tone and plasma vasopressin in this species.

Our findings indicate that inhibition of a discrete region in the caudal ventrolateral medulla prevents excitation of vasopressin-secreting neurones in the hypothalamus and produces excitation of vasomotor neurones in the spinal cord. Excitation of the same medullary region has the converse effect. The identity of the neurones responsible for the vasomotor effects remains an open question. Projection areas of A1 cells do not include the spinal cord (Blessing, Goodchild, Dampney & Chalmers, 1981; Westlund, Bowker, Zeigler & Coulter, 1983), so that these cells could only influence spinal vasomotor centres by an indirect, presently unknown, route. In contrast, the well-documented hypothalamic projection of A1 neurones means that these are the cells likely to be responsible for the regulation of plasma vasopressin. Over 80%, and possibly all, of the neurones in the caudal ventrolateral medulla with direct projections to the hypothalamus belong to the A1 group (Sawchenko & Swanson, 1982; Blessing, Jaeger *et al.* 1982). The studies of Day & Renaud (1984) and Day *et al.* (1984) show that 6-hydroxydopamine-induced destruction of noradrenergic nerve terminals in the supraoptic and paraventricular nuclei abolishes neuroexcitation of the neurosecretory neurones produced by stimulating the caudal ventrolateral medulla. Moreover, interruption of noradrenergic axons in the pons interferes with secretion of vasopressin in response to haemorrhage (Lightman, Todd & Everitt, 1984). Finally, application of noradrenaline to the supraoptic nucleus releases vasopressin into the circulation, apparently by activation of an alpha 1 adrenoceptor (Milton & Paterson, 1974; Urano & Kobayashi, 1978; Willoughby, Jervois, Menadue & Blessing, 1985).

Our results do not exclude the possibility of alternative inputs to the A1 cells. Bisset & Chowdrey (1984) emphasize the importance, for vasopressin secretion, of a nicotinic receptor located on neurones in the caudal ventrolateral medulla. These authors propose that this receptor is located on cholinergic cells which project from the caudal ventrolateral medulla to the hypothalamus. However, available anatomical studies show that the only cholinergic neurones in the region belong to the vagal preganglionic group in the nucleus ambiguus (Kimura, McGeer, Peng & McGeer, 1981; Armstrong, Saper, Levey, Wainer & Terry, 1983). Furthermore, the hypothesis is difficult to reconcile with the results of the 6-hydroxydopamine experiments described above. Because A1 noradrenergic neurones appear to excite the neurosecretory cells, the

results of Bisset and Chowdrey could equally well be explained by postulating that the nicotinic receptors are on A1 cells.

Alternative inputs to the vasopressin-secreting cells are known to come from the median preoptic nucleus, with connexions from the circumventricular organs (Miselis, Shapiro & Hand, 1979). Circulating angiotensin is believed to activate neurones in the circumventricular organs but evidence suggests that this system plays little part in the release of vasopressin in response to haemorrhage (Wang *et al.* 1983; Feuerstein, Johnson, Zerbe, Davis-Kramer & Faden, 1984). The A2 noradrenergic

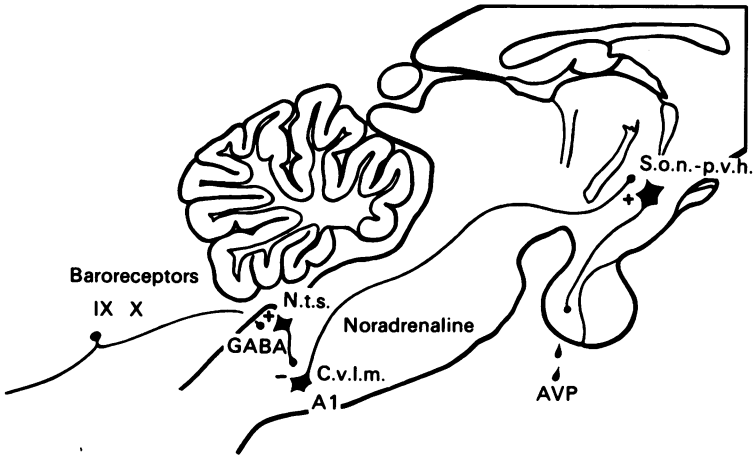

Fig. 5. Schematic outline of the hypothesized central neural pathways and transmitter agents mediating the baroreceptor-initiated secretion of vasopressin. The scheme is not intended to exclude alternative projections, either inhibitory or excitatory, from the nucleus tractus solitarius to the A1 cells. Abbreviations: n.t.s., nucleus tractus solitarius; s.o.n.-p.v.h., supraoptic and paraventricular nuclei.

neurones, within the nucleus tractus solitarius, project directly to the hypothalamus, but not to the vasopressin-secreting neuroendocrine cells (Sawchenko & Swanson, 1982).

Thus the central pathway for baroreceptor-mediated secretion of vasopressin may be that shown in Fig. 5, as first suggested, in more general terms, by Feldberg & Rocha E Silva (1981). The complete prevention of vasopressin release observed in the present study implies that neurones in the caudal ventrolateral medulla are essential, excitatory elements in the pathway transmitting the baroreceptor-initiated vasopressin–secretion reflex from the medulla to the hypothalamus. The A1 noradrenergic cells are likely to be the neurones involved.

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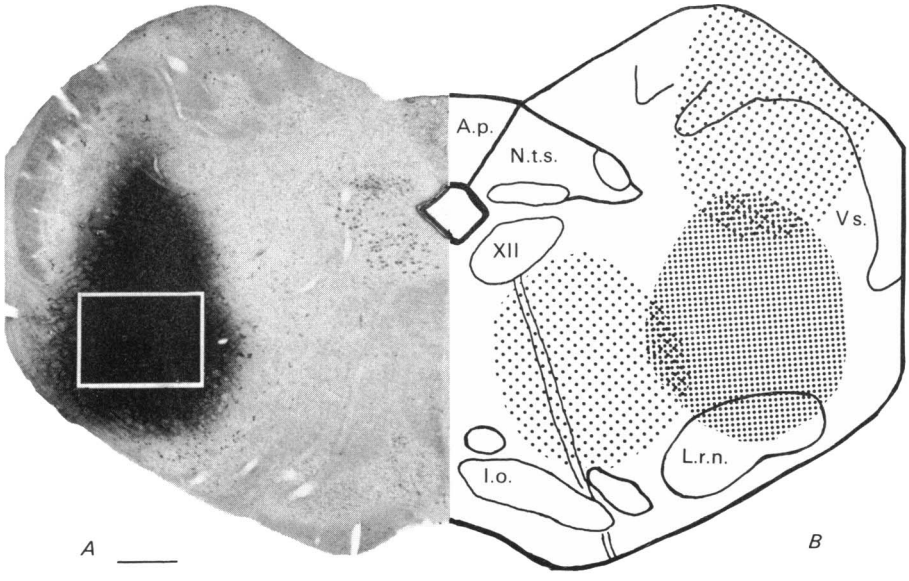
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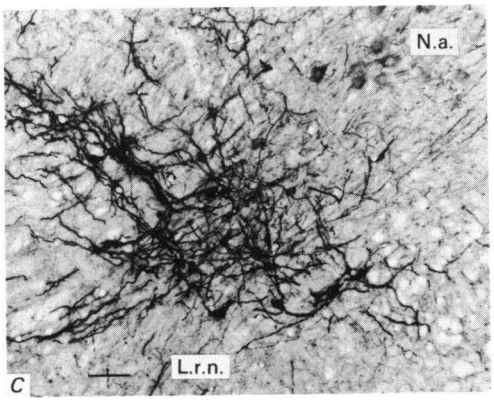
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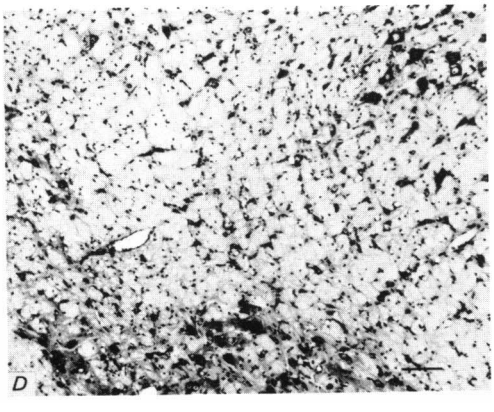
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EXPLANATION OF PLATE

A, photomicrograph of Nissl stain of rabbit caudal ventrolateral medulla. The rostro-caudal level, 1 mm caudal to the anterior border of the area postrema, is at the level of the exiting hypoglossal nerve rootlets and thus corresponds with the caudal, nicotine-sensitive, depressor area described in the cat. The dark area is horseradish peroxidase reaction product, indicating the centre of the injection site and the direction of spread. The size of the area depended on the concentration of horseradish peroxidase included in the injection. Bar = 0·6 mm. *B*, diagrammatic representation of the experimental site in the caudal ventrolateral medulla and the two control injection sites, dorsal and medial to the experimental site. *C*, photomicrograph of tyrosine hydroxylase immunoreactive cell bodies and dendritic processes located in the region shown by the rectangle in *A*, demonstrating that A1 neurones are located in the centre of the injection site. Bar = 100 μm. *D*, photomicrograph of the same region from a different animal, stained for Nissl substance. By comparing *C* and *D* one can verify that the A1 neurones occur in the region between the caudal cells of the nucleus ambiguus and the lateral reticular nucleus. Bar = 100 μm. Abbreviations: A.p., area postrema; I.o., inferior olive; L.r.n., lateral reticular nucleus; N.a., nucleus ambiguus; N.t.s., nucleus tractus solitarius; Vs., spinal nucleus of the trigeminal nerve; XII, hypoglossal nucleus.