Pressor Response to Electrical and Chemical Stimulation of Nucleus Raphe Dorsalis in the Cat


SUMMARY The dorsal raphe nucleus was stimulated electrically and chemically in 57 cats with blood pressure being measured by an arterial catheter. Blood pressure rose by a mean of 119.5 ± 8.9% on stimulation of the dorsal raphe nucleus at a frequency of 200 sec⁻¹. This pressor response was frequency-dependent over the range 0.2 to 200 sec⁻¹ and the site of origin was localized to the dorsal raphe nucleus. The pressor response could also be obtained by the injection of D,L-homocysteic acid into the nucleus, and is therefore likely to be due to activation of cell bodies rather than axons of passage. The response was blocked by high spinal cord section section but was not affected by bilateral clamping of the adrenal glands or supracollicular decerebration. The pressor response was significantly reduced by pretreatment of the cats with parachlorophenylalanine (PCPA) and abolished by the alpha receptor blocker phentolamine, suggesting that both serotonergic and noradrenergic synapses are involved.

HYPERTENSION is the most important factor in the genesis of stroke and the mechanism of neurogenic hypertension is of importance to any clinician dealing with cerebrovascular disease. Significant physiological evidence is available to support the contention that the dorsal raphe nucleus and the transmitter serotonin are involved in cardiomodulatory roles. Smith et al⁷ have shown that serotonin synthesis is increased in the hypothalamus, brainstem and spinal cord of spontaneously hypertensive rats but any attempt at altering central serotonin synthesis with agents such as para-chlorophenylalanine (PCPA) has had variable effects on blood pressure depending on the species, initial physiological state (whether hypertensive or not), and on the route of injection of the drugs. Lambert et al⁶ have shown that intraventricular injection of serotonin leads to an increase in rat blood pressure that is blocked by LSD, a serotonin antagonist, but not by pretreatment with 6-hydroxydopamine, in contrast to the depressor response reported by Tadepalli et al.⁵ Two groups of investigators have examined the effect of the electrical stimulation of the rat midbrain raphe nuclei on systemic blood pressure.⁴,⁷ Both groups observed an increase in systemic blood pressure with no effect on heart rate, while Kuhn et al⁴ also found that the pressor response was increased and prolonged by fluoxetine, a serotonin uptake antagonist. Depletion of brain serotonin with para-chlorophenylalanine significantly attenuated the pressor response to electrical stimulation of the median raphe nucleus⁴ and both the median and dorsal raphe nucleus. This response was also diminished by the injection of the serotonin antagonist 2-bromolysergic acid diethylamide (BOL) into the anterior hypothalamic/preoptic area. Gillis et al⁸ described, in addition to a pressor response, an increase in heart rate, pupillary dilatation and piloerection after stimulation of the dorsal raphe nucleus in the cat. The pressor response could be blocked by phentolamine.

Despite these investigations certain important questions are unanswered. Is the pressor response to electrical stimulation of the midbrain dorsal raphe nucleus restricted to the nucleus or is it a nonspecific effect of midbrain stimulation? Is the pressor response due to cell body activation or merely an excitation of fibers of passage? Can it be blocked or modulated by serotonin depleters if central catecholamine mechanisms are left intact?

Methods

General Surgical Procedures

Eleven male and 46 female cats (table 1), average weight 2.7 ± 1.0 kg (mean ± SD), were anesthetized with a mixture of α-chloralose (20 mg/kg⁻¹) and urethane (500 mg/kg⁻¹) administered intraperitoneally. Polyethylene catheters were inserted into a femoral vein for the injection of drugs and into the femoral artery to monitor systemic blood pressure. The animals were intubated, paralysed with gallamine, and artificially respired so as to maintain a constant end-expiratory CO₂ level of 4%. Body temperature was kept constant with the aid of a heating blanket. The cats were mounted in a stereotaxic apparatus, a skin flap turned, a 2 cm² midsagittal window was placed in the calvarium and the dura reflected for introduction of the electrode.

Electrical and Chemical Stimulation

The dorsal raphe nucleus is located at the following co-ordinates based on the atlas of Berman:⁹ P 0.2, H −1.0, L 0.0. A series of points in the surrounding brainstem were then stimulated. A bipolar stainless steel electrode (Rhodes NEX-100), insulated except for 0.5 mm at the tip, was used to deliver paired opposite-polarity pulses (0.2 to 200 sec⁻¹, 500 μA, 250 μsec duration, 500 μsec separation) from two Devices 2433 isolated stimulators driven by a Grass S88 stimulator. Each period of stimulation lasted for 15 seconds.

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The stimulus was designed to excite a 1 mm diameter sphere of the brainstem.\(^\text{10}\)

The results of electrical stimulation were compared with those of chemical stimulation by L-glutamate and DL-homocysteic acid (DLH), both having nonspecific excitatory effects on most central neuronal cell bodies.\(^\text{11-13}\) Using the same stereotaxic co-ordinates as for electrical stimulation and the microinjection method of Goodchild et al.,\(^\text{14}\) 120–240 nl of 1M L-glutamate (sodium salt), DL-homocysteic acid or saline vehicle were delivered per injection from glass micropipettes.

### Blood Pressure Monitoring

A Statham pressure transducer (type p23AC) was used to monitor systemic blood pressure via the femoral arterial catheter, with the signal being passed to a custom-made monitor so that blood pressure could be constantly viewed. A signal conditioning device provided analog outputs for chart recording and for feeding the analog-digital converter of a Z-80 based microcomputer. A Fortran (Microsoft) programme monitored data acquisition, printed out results (changes in physiological variables measured), and stored the data on floppy disc. On-line data acquisition consisted of 25 seconds of control observations followed by stimulation and recording until blood pressure returned to prestimulation levels. The entire system has been more fully described elsewhere.\(^\text{15}\) BASIC (Microsoft) programmes were written to average all measurements. The "control" response is taken as the frequency-response at 50 sec\(^{-1}\) at each of these points is recorded unless otherwise indicated. The frequency-change in blood pressure with DRN stimulation was performed on an X-Y plotter using a specially written C program.

### Histology

Placement of electrodes was verified histologically. The stimulus site was marked using the Prussian blue method, in which Fe\(^{+++}\) ions are deposited into the brain by passing a 2.5mA direct current through the tip of the stimulating electrode for 30 seconds, and then perfusing the animal with 10% formalin and a mixture of potassium ferrocyanide and ferricyanide. For the purpose of localizing the microinjections, the dye fast-green was mixed into the solution with either of the amino acids. The fast-green could be identified in subsequent histological sections and verified the placement of the injections to be correct. The brains were then sectioned and stained with neutral red. Corrections were made if necessary between actual and calculated stereotaxic placement.

### Pharmacology

To block peripheral \(\alpha\)-receptors 5 mg/kg\(^{-1}\) phentolamine was injected intravenously into 4 cats. An injection of 2 \(\mu\)g/kg\(^{-1}\) noradrenaline was used to check that alpha blockade was complete during stimulation. To deplete central serotonin levels selectively while protecting noradrenergic neurons, an intraperitoneal injection of 25 mg/kg\(^{-1}\) desmethylimipramine, followed 60 minutes later by 500 mg/kg\(^{-1}\) parachlorophenylalanine (PCPA), was performed 72 hours before stimulation of the dorsal raphe nucleus stimulation. The cats thus treated showed no changes in mood nor appeared in any distress, although they were less inquisitive.

### Statistics

Results are expressed as percentage change from prestimulus resting blood pressures to eliminate the inter-cat variability in absolute blood pressure measurements. The "control" response is taken as the percentage change in blood pressure with DRN stimulation in an otherwise intact animal. The frequency-response data obtained in this series of experiments were initially examined using a three-way analysis of variance,\(^\text{16}\) employing the response of each cat, frequency of stimulation and treatment (e.g. pre- and post-spinal cord section), as the three components of variation. Subsequent analyses were carried out on data shown to be significant by this procedure. A quadratic polynomial regression was carried out on the mean blood pressure response at each frequency of stimulation i.e. frequency versus mean blood pressure change. The subsequent coefficients of these regressions were tested against each other (e.g. control versus spinal cord section), to determine if frequency-response curve differences were significant. All tests were considered to be significant at the \(p < 0.05\) level unless otherwise indicated.

### Results

#### Localization

In 57 cats mean arterial blood pressure was 109 ± 2.6 mm Hg. The area of the brainstem about the dorsal raphe nucleus eliciting a pressor response was mapped by electrical stimulation. This response was well localized to the dorsal raphe nucleus (fig. 1) and did not diminish during the period of the experiment. Penetrations with the electrode were made at combinations of antero-posterior (AP- + 1.0, + 0.5, 0.0 and -1.0 mm) and lateral (L-0.0, 0.5, 1.0 and 1.5 mm) sites and at heights from + 5.0 to − 7.0 at 0.5 mm intervals all measured from cat stereotaxic zero.\(^\text{9}\) The results of stimulation at 50 sec\(^{-1}\) at each of these points is recorded in figure 2. Each point representing the mean of between 4 and 12 stimulations. Injection of DLH at 1 mm or 2 mm lateral or 2 mm anterior or posterior to the
dorsal raphe nucleus had no effect on blood pressure, thus demonstrating the localization of the response to the cell bodies of the raphe nucleus. Some injections did not enter the brainstem proper but were placed in the roof of the aqueduct and resulted in no response.

The mean maximum increase in blood pressure to electrical stimulation in the intact cat (designated "control" response) was 119.5 ± 8.9% at 200 sec⁻¹, and was frequency-dependent over the range 0.2 to 200 sec⁻¹ (fig. 3). This response could be reproduced by injection of 120-240 nl of DL-homocysteic acid (DLH) into the dorsal raphe nucleus (fig. 4) but could not be reproduced by the injection of L-glutamate. The mean maximum blood pressure rise after DLH injection was 41.7 ± 6.5% (n = 8) while, with L-glutamate injection, the mean maximum change in blood pressure was 6.5 ± 0.45% (n = 10). During control stimulations piloerection and pupillodilation were observed although no changes in heart rate were detected.

Surgical Manipulation

Spinal cord section at the C1/C2 level led to an immediate drop in systemic blood pressure which recovered well with the aid of intravenous fluid (15-20 mls Hemaccel). The pressor response to dorsal raphe nucleus stimulation was completely blocked after spinal cord section (fig. 4).

Bilateral clamping of the hila of the adrenal glands resulted in no significant change in resting blood pressure and did not block the pressor response to DRN stimulation (fig. 5). The mean maximum response after bilateral clamping of the adrenal hila was not significantly reduced from control levels (77.7 ± 12.7%, n = 3; Tₜ₁ = 1.76).

Decerebration

In 5 cats supra-collicular decerebration, which included removal of the hypothalamus, had no significant effect on the pressor response to dorsal raphe nucleus stimulation (tₜ₃ = 0.60). Although mean arterial blood pressure was lower in these animals the mean maximal response to stimulation was large (133 ± 8.7%, n = 5).

Pharmacology

In 4 cats 5 mg/kg⁻¹ phentolamine was administered intravenously. Alpha blockade was checked by administration of 2 µg/kg⁻¹ noradrenaline, and was complete throughout the experiment. The pressor response to dorsal raphe nucleus stimulation was completely blocked by phentolamine (fig. 4).

In 4 cats pretreated with desmethylimipramine and PCPA mean blood pressure was 115 ± 3.7 mm Hg which did not differ significantly from controls (tₜ₃ = 0.18). In PCPA-treated cats stimulation of the dorsal raphe nucleus resulted in a mean maximum increase in blood pressure of 14.6 ± 5.6% which was significantly less than that seen in control animals (F₀₀₁;₁₂₇ = 70.02; fig. 3) whereas stimulation of the locus coerules evoked a pressor response that was not significantly different from that previously reported (tₜ₅ = 0.21; 15). This confirmed the integrity of the central noradrenergic systems, and thus demonstrated the specificity of the depleting affect of PCPA on the dorsal raphe nucleus.

Discussion

Stimulation of the midbrain has delineated the origin of a specific pressor response to the dorsal raphe nucleus. The response is well localized antero-posteriorly, laterally and dorso-ventrally, which would strongly suggest that the nucleus itself was the site of origin of the pressor response. After the maximum pressor response was delineated, further mapping at this level was performed with DLH. The lack of any response lateral to the nucleus provided strong evidence that the cell bodies of the dorsal raphe were responsible for the pressor response we have reported here. The lack of effect of injections into the cerebral aqueduct or into areas surrounding the DRN is clear evidence that the pressor response observed is due to local excitation of the nucleus not to any distant structure. Although there are no studies on the diffusion potential of DLH and its sphere of excitation, this question is best addressed by careful localisation of the physiological response as in the present study. The demonstration that the injection of DL-homocysteic acid into the DRN causes a large pressor response indicates that the origin is from cell bodies not axons of passage.

Interestingly, L-glutamate has very little effect when injected into the dorsal raphe nucleus. Krnjevic¹⁰
Electrical Simulation of the Midbrain

Blood Pressure Response

Cat

A 0.5

FIGURE 2. Cross-sections of the brain of the cat at various positions in the region of the dorsal raphe nucleus both anterior (A) and posterior (P) to stereotaxic zero and lateral (L) to the midline. Each point represents the mean percentage change in blood pressure (BP) from control for the cohort of animals (4–12 cats) electrically stimulated at a particular site. The ordinate of each graph represents the height of the point above and below stereotaxic zero in millimetres (mm). The main area of the dorsal raphe and raphe centralis superior is shaded. See figure 1 for anatomical details.

has listed DL-homocysteic acid as being in general a stronger excitatory amino acid than L-glutamate. Hosli and Tebecis12 have also shown a strong excitation of reticular activating neurons by DL-homocysteic acid in the absence of any effect of L-glutamate. The reason for this difference may reside in the difference in uptake mechanisms for each substance. L-glutamate is actively taken up by glial cells18 and neurons,19 whereas no specific mechanism for DL-homocysteic acid has been described. Since the dorsal raphe nucleus is relatively diffuse, it is conceivable that much of the L-glutamate is taken up before it can activate the whole nucleus, while DL-homocysteic acid is permitted to spread throughout the whole nucleus and thus activate much more of it, reproducing the pressor response to electrical stimulation more closely. Activation of the pressor response with DLH has a longer time course than does electrical stimulation which supports the notion that the DLH has to diffuse through the nucleus to have its eventual effect.

Sympathetic Nervous System

The pressor response is clearly mediated via spinal mechanisms since it is abolished by high spinal cord section. Although this may seem obvious, Lambert et al4 could not block the pressor response to intraventricular serotonin with 6-hydroxydopamine, suggesting some non-sympathetic mediation of the pressor response. The time course of the response, an almost immediate increase in blood pressure with electrical stimulation, also argues for neurogenic mediation. Blockade of the response with phentolamine was not surprising since it is mediated through spinal mechanisms, presumably the sympathetic nervous system.

FIGURE 3. The effect of pre-treatment with PCPA on the pressor response to DRN stimulation. Frequency response curve for percentage increase in blood pressure with stimulation of the dorsal raphe nucleus both before (Control) and after PCPA. Percentage change in blood pressure is represented on the ordinate and frequency of stimulation on the abcissa.
PRESSOR RESPONSE TO DORSAL RAPHE NUCLEUS STIMULATION

Gillis et al. has previously demonstrated blockade of this response but his observations include a tachycardia which we and others have not seen.

Adrenal Gland

The data presented here is evidence that the adrenal gland is not involved in the immediate pressor response to dorsal raphe nucleus stimulation. This conclusion applies to the immediate pressor response only, since we are investigating further any relationship between the adrenal gland and a delayed (post-stimulus) carotid constriction mediated by the dorsal raphe nucleus in the cat.

Decerebration

Decerebration of the cats in this series of experiments was supra-collicular and involved removal of the hypothalamus. The hypothalamus has long been implicated in cardiovascular control, and especially in sympathetically mediated responses. It was therefore of considerable interest to demonstrate that the pressor response is not dependent on an intact hypothalamus or for that matter any ascending pathway, since they were all removed. The pressor response must therefore be mediated by brainstem connections of the dorsal raphe nucleus with appropriate pressor areas in the lower brainstem.

PCPA

It was considered important to test whether the pressor response was due to serotonin-containing cells or to other cells since only 60% of the cells in the dorsal raphe nucleus contain serotonin. Because the administration of PCPA, a central serotonin-depleting substance, prior to the experiment significantly reduces the pressor response we conclude that the response depends upon a serotonergic synapse. We have employed desmethylimipramine in this series of experiments to protect central catecholaminergic systems from any non-specific affects that may confuse the interpretation of our data. Alternating stimulation of the locus coeruleus and dorsal raphe nucleus demonstrated clearly that this approach leaves central noradrenergic mechanisms intact, since the normal response to electrical stimulation of the locus coeruleus that we have observed was reproduced after PCPA administration. The serotonergic synapse responsible for the pressor response presumably projects from the dorsal raphe nucleus to the lower brainstem and thence to the spinal cord.

In conclusion, we have shown the pressor response to dorsal raphe nucleus stimulation to be well localized to the nucleus itself, to be reproduced by micro-injec-

FIGURE 4. Computer processed plots showing blood pressure responses to dorsal raphe nucleus stimulation in a subpopulation of cats after certain manipulations. The ordinate represents percentage change in blood pressure, while the abscissa is time with the point and duration of stimulation marked (solid line). The control response as well as responses after DL-homocysteic acid (DLH), L-glutamate (glutamate), phentolamine and after spinal cord section are shown. On the relevant traces (control, phentolamine and spinal cord section), solid lines represent the mean response from several animals and the dashed lines are standard errors of those means. The frequency of electrical stimulation was 50 sec⁻¹. Standard errors are not shown for the individual traces after chemical stimulation for technical reasons related to the recording of and averaging of this data by computer.
tion of DL-homocysteic acid, making it likely to be due to excitation of cell bodies rather than fibers of passage, and to be mediated via a serotonergic synapse through spinal, α-adrenergic mechanisms to the periphery. Adrenal gland activation does not play a necessary role in this pressor response, nor does the hypothalamus. Since serotonin pathways are known to project diffusely to cerebral cortex from the brainstem, and downwards from the brainstem as part of the endogenous pain control system, it is possible that they also play a part in mediating the pressor response in alert or arousal states.

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