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Areas in the amygdala necessary to the operation of the vagosympathetic pressor reflex

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Summary. Lesions of portions of the basal and cortical amygdaloid nuclei and the stria terminalis reversed or abolished arterial pressor responses to ipsilateral centripetal vagal stimulation (CVS). Destruction of these particular limbic structures in the rat did not affect cardiopulmonary responses to CVS.

The association of centripetal vagal stimulation (CVS) with reflex alterations in the cardiovascular and respiratory systems in bilaterally vagotomized dogs, cats, rabbits, rats, guinea-pigs, and cold blooded animals has been studied by a number of investigators over a considerable period of time. A variety of cardiopulmonary reflexes have been elicited from these species, but the arterial pressor response remains one of the best documented indices of visceral afferent activity[1-5]. In addition to the circulatory responses to electric CVS, various reflexes affecting the canine gastrointestinal tract have been described as occurring in response to the same stimulus parameters as those which are adequate to produce the arterial pressor response[6-9]. It is generally agreed from observations with dogs that the essential reflex arc involved during CVS consists of an afferent limb running up the vagus and an efferent limb running in the spinal sympathetic nerves[10-13]. Of course the definition of a reflex includes at least one synapse in the central nervous system. Therefore, a complete description not only includes the limbs and the neurohumors involved, but the central connection as well. At this point it may be well to indicate that the medulla has long been associated with the integration of most autonomic visceral functions. However, there are experimental indications in the literature that forebrain regions may also be involved in the integration of cardiovascular reflexes[14,15]. One of the forebrain regions most closely related to cardiovascular functioning is the amygdala[16-18]. Thus, the purpose of these experiments was to determine if the arterial pressor response involves an amygdalar pathway.

Materials and methods. Acute experiments were performed on 19 adult male and female Wistar rats (210-440 g b.wt) anesthetized with an i.p. injection of urethan (1.7 g/kg b.wt). Respiratory activity was recorded as a function of pressure changes which occurred in a side arm of a surgically inserted tracheal cannula. A polyethylene catheter was inserted into either the femoral or carotid artery for the measurement of blood pressure. A lead II ECG was taken in all cases. Monitored parameters were recorded on a Grass Model 7 polygraph. In all experiments, the cervical vagi were exposed, divided, and the central ends were prepared for unilateral electrical stimulation. CVS was accomplished with a Grass SD-9 stimulator. The stimulator delivered a monophasic square wave pulse (2-7 V, 60 Hz, 10 msec), which was applied for a 10-sec duration followed by a rest interval of at least 60 sec. Absolute current flow was measured using voltage drop determination across a precision resistor of known value in series with the stimulating bipolar electrodes. Centripetal stimulation of either vagus with the specified electrical stimulation was sufficient to elicit the desired end-point of response: a rise in arterial blood pressure and apnea spuria. In each animal, electrolytic lesions were produced in the region of the amygdala, ipsilateral to the CVS arterial pressor response, by passing a

Comparison of MAP prior to and during CVS before and after lesioning of the amygdalar nuclei and surrounding structures

Locations of amygdalar lesions		MAP (mmHg) Pre lesion			Post lesion		
		Prior to CVS	During CVS	Change	Prior to CVS	During CVS	Change
Lesion sites in panel A of figure 2	(11)	97±6	128±5	+30±6	99±6	122±5	+24±6
Lesion sites in panel B of figure 2	(8)	102±6	133±6	+31±4	107±4	96±5*	−11±8*

All values are means±SEM. MAP, mean arterial pressure; CVS, central vagal stimulation; number of animals lesioned in parentheses; * $p<0.01$.

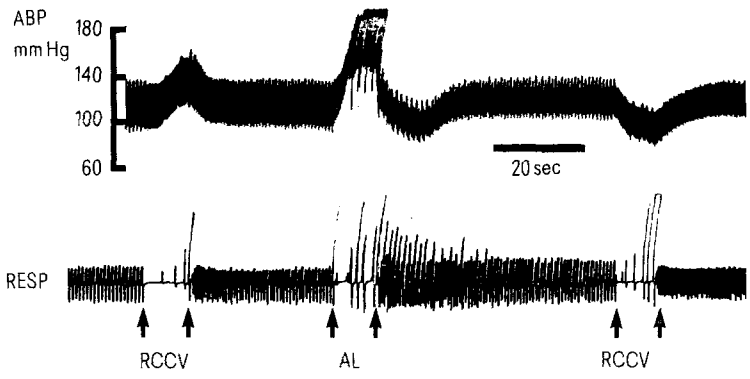

Fig. 1. Effects of lesioning the amygdala on the cardiovascular and respiratory responses to CVS. ABP, arterial blood pressure; RESP, respiratory side pressure; RCCV, right centripetal cervical vagal stimulation; AL, amygdalar lesion. Stimulation parameters: 5 V, 60 Hz, 10 msec. Current was applied between arrow pairs.

direct current of 0.25 mA for 10 sec, via a Grass constant current lesion generator, through a bipolar 36-gauge enameled nichrome alloy wire (27.6 Ω/ft) located stereotaxically using the rat brain atlas of König and Klippel[19]. Polygraphic tracings of CVS-induced arterial pressor responses were obtained before and at stated intervals after the lesioning procedures in each group of rats. The minimum diastolic and maximum systolic pressures were measured from polygrams just prior to CVS and for an identical period corresponding to the maximum arterial pressure change during CVS. Mean arterial pressure (MAP) was calculated as diastolic plus one-third of the pulse pressure. Differences in MAP were inferred by a statistical comparison of mean values at a significance level of 1% using a paired Student t-test[20]. At the end of each experiment the brain was removed, formalin fixed, frozen sectioned, and stained with cresyl-echt violet[21] in order to verify the location and extent of the lesion.

Results and discussion. In all bilaterally vagotomized rats, centripetal electric stimulation (0.4–0.6 mA) of either vagus nerve at the cervical level always resulted in an increase in MAP (table), with little or no change in heart rate but near cessation of respiration during the stimulus interval followed by an increase in rate of breathing (figure 1). In figure 1, it can be readily seen that the CVS-induced arterial pressor response was not only completely abolished by a lesion in the amygdala but literally converted to a depressor response. Figure 1 reveals that an exaggerated arterial pressor response could be produced by the stimulation of the amygdalar site which occurred during the production of the electrolytic lesion. This figure also reveals that destruction of this particular amygdalar structure did not affect the respiratory response to CVS. It is interesting to note, that after lesioning the amygdala the resting MAP was not altered (figure 1 and table). This absence of a role of the amygdala in the maintenance of resting MAP has previously been reported[22] and is in marked contrast to the hypothalamus which when destroyed does lower the MAP[23]. As can be seen in figure 2B, lesions of portions of the basomedial, basolateral and cortical nuclei of the amygdala eliminated the arterial pressor response to CVS. On the other hand, lesions in the contiguous areas of the amygdala (figure 2A) had no effect on the pressor response to CVS. It must be emphasized that while our lesion-defined area delineated in figure 2B neither corresponds to the traditional subdivisions of the amygdala (basolateral and corticomedial) based on embryological and comparative anatomical studies[24], nor to any known functional subdivision based on electrical stimulation studies[14,18,24] it does correspond to the primary nuclei receiving and supplying fibres to the stria terminalis (ST)[25–27], one of the main fibre tracts of the amygdala. Interestingly enough, the ST has been identified as a fibre tract mediating the hypertension due to appropriate stimulation of the amygdala[14]. Similarly we have found that the arterial pressor response to CVS could also be completely abolished by transections of the stria terminalis 4 mm anterior to the frontal zero plane[19]. Again the respiratory response persist-

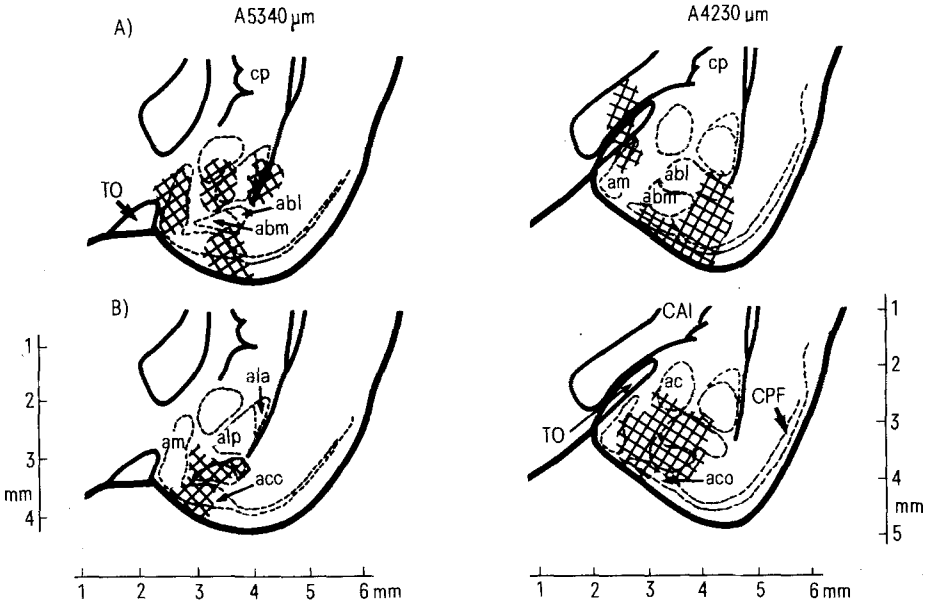

Fig. 2. Diagrams of transverse sections of rat brain showing the locations of lesions placed within the amygdala. The crosshatching represents single lesions or composites of many lesions. *A* Lesions within the amygdala having no effect on the pressor response during CVS. *B* Lesions within the amygdala which eliminated the pressor response during CVS. Nuclei of the amygdala: abl, basolateral; abm, basomedial; ac, central; aco, cortical; ala, anterolateral; alp, posterolateral; am, medial. CAI, internal capsule; cp, caudate putamen; CPF, piriform cortex; TO, optic tract. Numbers above the sections give rostral-caudal coordinates of section while the horizontal and vertical coordinates are given in mm from the sagittal and frontal zero planes respectively[19].

ed (unpublished data). In sum, it appears likely that certain regions of the amygdala may be involved in the vagosympathetic pathway. Such knowledge is of paramount importance in consideration of the physiological stimulus for the CVS arterial pressor response.

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Opposing temperature responses to intrahypothalamic injections of 5-hydroxytryptamine in the pigeon exposed to cold

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Summary. 5-Hydroxytryptamine injected into posterior and anterior parts of the pigeon hypothalamus evoked a short lasting hyperthermia or hypothermia, respectively. Variable responses obtained within the same brain region suggest the existence of different 5-HT systems, even in rather limited hypothalamic areas.

Although the anterior hypothalamus is generally thought to represent the main site of regulation of body temperature (T_b), structures in the posterior aspects have also been found to contain neurones controlling responses especially against cold[1]. While the pigeon brain stem has been shown to be rather insensitive to thermal stimulations[2], cerebral injections of putative neurotransmitters elicit responses suggesting thermoregulatory role on them[3-7]. In the pigeon, injection of 5-hydroxytryptamine (5-HT) into the anterior hypothalamus has been demonstrated to induce a slight hypothermia at an ambient temperature (T_a) of 15 °C[5]. At certain sites, however, a rise of T_b following 5-HT has been reported[8]. To ascertain the dual effect of 5-HT on T_b, injections were made into the posterior hypothalamic region, and the results compared with those obtained from experiments at more rostral locations.

Materials and methods. Using pentobarbital anesthesia, a guide cannula was implanted stereotaxically[9] into the hypothalamus of 9 domestic pigeons, weighing 260–330 g, 5.0–5.6 mm (modified coordinates[7,9]) anterior to the inter-aural line (posterior hypothalamus). The cannula extended 9.0–11.0 mm below the skull surface. 1 week was allowed for recovery from surgery.

During the experiments, pigeons exposed to T_a 6 °C were injected with a 5-HT solution in a volume of 1.0 µl containing 10 µg of salt (5-hydroxytryptamine creatinine sulphate, Merck) dissolved in distilled water. Methods of measurements of oxygen consumption, body and foot temperatures and shivering have been described earlier[10,11].

Additionally, results of similar injections using stereotaxic coordinates 6.5–8.0 mm anterior to the inter-aural line (anterior hypothalamus) were compared with those obtained from more caudal locations. Injections into the rostral aspects were made earlier in a different context but under similar conditions in our laboratory.

Results and discussion. Contrary to the results obtained earlier[5], injection of 10 µg 5-HT into the posterior hypothalamus did not exclusively induce hypothermia. Instead, a short lasting T_b increase of 1.0±0.27 °C ($\bar{x} \pm SE$) was evoked in 7 pigeons. The latency for the onset of temperature rise was 1–2 min, and the peak was achieved within about 20 min of injection. Hyperthermia was often accompanied by a substantial increase in the strength of shivering, and an increase of 14.2% on the average in the oxygen consumption. The results were reproducible as demonstrated by repeating the injection during the same experimental session. In 2 pigeons, the injection of 5-HT produced a biphasic temperature response: the immediate small rise (0.2 and 0.5 °C) with the peak within less than 10 min was followed by a hypothermia reaching nadir within 40–50 min.

Most of the injections (14 out of 22 made on equal number of birds) into a more anterior portion evoked hypothermic responses inducing T_b fall of 0.7±0.11 °C ($\bar{x} \pm SE$) thus confirming the previous report[5]. Injections into 5 pigeons produced increased shivering, however, and were followed by a rise of T_b (0.6±0.19 °C, $\bar{x} \pm SE$). In 2 birds, the injections were practically without effect, and in 1 case a biphasic response (T_b changes of +0.7 and −2.0 °C) was recorded.

Despite the previous conclusion that the concept of noradrenaline (NA) and 5-HT acting as an antagonistic pair in