Distribution of Choline Acetyltransferase in Cerebral and Extracerebral Cranial Arteries of the Cat
Its Relationship to Neurogenic Atropine-Sensitive Dilation

J. A. Bevan, G. M. Buga, V. M. Florence, A. Gonsalves, and A. Snowden
From the Department of Pharmacology, School of Medicine and Brain Research Institute, University of California, Los Angeles, California

SUMMARY. Choline-acetyltransferase (ChAT) activity was surveyed in segments of cranial arteries—both cerebral and extracerebral—from the cat. High levels were found in pial arteries, both cerebral and cerebellar, and in the arteries to salivary glands, tongue, and nose. Intermediate levels were found in the external and internal maxillary arteries and many of their branches. Enzyme levels in the arteries supplying the head—common carotid, vertebral, and in several systemic arteries and veins and also the lingual vein—were probably not significant. Only those vessels that have higher ChAT contents show capacity for neurogenic vasodilation. The dilation of segments of a number of these arteries, the basilar, middle cerebral, lingual, and internal maxillary, is reduced significantly by atropine (5 × 10^{-7} M). ChAT activity did not correlate with vessel norepinephrine content. The data may be interpreted as defining a functional vasodilator system to the head encompassing both cerebral and extracerebral arteries that depends in part on a functional cholinergic link involving a muscarinic receptor. It is separate from the adrenergic outflow. The tissues supplied by vasculature receiving this type of innervation are of ectodermal origin. (Circ Res 50: 470-476, 1982)

AS PART of our investigation of the mechanism of neurogenic dilator innervation of cerebral vessels, two biochemical indices of cholinergic mechanisms—choline-acetyltransferase (ChAT) and high affinity choline uptake—were measured and found to be high in cerebral arteries of three species, the cat, dog, and rabbit (Florence and Bevan 1979). This was unexpected, inasmuch as functional in vitro studies after sympathetic inactivation, in which classical pharmacological analyses were utilized, had failed to reveal evidence of a functional cholinergic link between neural activation and vascular relaxation in these vessels (Lee et al., 1978). High levels of these biochemical indices, generally considered to be specific for a cholinergic mechanism, are thought to incriminate rather unequivocally a functional cholinergic link (Yamamura et al., 1974).

A nonsympathetic neural outflow to blood vessels resistant to blockade by antimuscarinic drugs is reminiscent of the characteristics of vasomotor systems to other tissues of the head—including salivary glands, nose, and tongue (Erici and Uvnas, 1952; Gautick, 1970; Anggard, 1974; Stjenschantz and Bill, 1980). These several observations raised the possibility that the biochemical indications of a cholinergic mechanism might not be unique to cerebral vessels, but might be found in other arteries supplying structures of the head, even though these have been reported to be atropine-resistant.

The evidence presented in this study indicates that ChAT is present in high amounts in a number of arteries of the cat cranial vasculature, both cerebral and extracerebral, in particular, in vessels to brain, tongue, salivary glands, and nose. That this enzyme may in some way or other be related to the dilator response is suggested by the finding that only those vessels with a significant level of this enzyme exhibited neurogenic dilation and that this dilation in a number of vessels is significantly reduced by atropine. ChAT levels are not related to an index of adrenergic innervation density—specifically, endogenous norepinephrine content. Mapping the geographical density distribution of a specific indicator of a putative transmitter in the arterial tree, in this case ChAT, and relating this to the distribution of the specific local neural response, atropine-modified dilation, is the approach adopted in this paper to study the mechanism(s) involved and the significance of the neural system.

Methods

Cats (2-4 kg) were anesthetized with sodium pentobarbital (50 mg/kg, iv). The entire brain with pial vessels attached, together with other arteries and veins to be examined, was removed rapidly and placed in gassed Krebs’ phosphate solution on ice for dissection with the aid of a dissection microscope. The composition of the Krebs’ solution was: NaCl, 115 mM; KC1, 4.75 mM; CaCl2, 1.3 mM; KH2PO4, 1.18 mM; MgSO4, 3.5 mM; sodium phosphate buffer, 15.6 mM (pH 7.4); and glucose, 12 mM (pH 7.4).

Blood Vessels Studied

The following arteries were assayed for ChAT: proximal and distal parts of common carotid, lingual and facial; the internal maxillary, proximal and distal to the rete; the
maxillary rete; proximal parts of sphenopalatine, infraorbital, superficial temporal, posterior auricular; arterial branches to parotid and sublingual glands; the pre-canal, canal, and atlas segments of vertebral; basilar, proximal parts of middle and posterior cerebral and posterior inferior cerebellar; the proximal part of the subclavian; and the radial near the wrist.

Segments of the cephalic and lingual veins were also assayed.

In addition, selected segments were also analyzed for their norepinephrine content and for study in vitro.

**ChAT Assay**

Iris and blood vessels were homogenized in Potter-Elvehjem glass grinders ( Kontes Glass Co.) in a homogenization solution containing NaCl, 300 mM; sodium phosphate buffer, 10 mM (pH 7.4); EDTA, 1 mM; and Triton X-100, 0.5% (vol/vol) (pH 7.4) (Fonnum, 1975). The volume of the homogenization solution was adjusted to give a tissue concentration of 1.5 mg/40 μl. The procedure was carried out on ice.

The radioenzymatic assay was adapted largely from Fonnum’s method (1969, 1975). The ChAT activity was determined in 5-ml centrifuge tubes (with ground glass stoppers). To the homogenate (40 μl), the following substrate mixture (10 μl) was added (final concentration per 60 μl): [1-14C]acetyl-coenzyme A 50–60 mCi/mmol (New England Nuclear), 0.20 mM; NaCl, 300 mM; sodium phosphate buffer, 50 mM (pH 7.4); choline chloride, 8 mM; EDTA, 10 mM; and eserine sulfate, 0.11 mM (pH 7.4). The [14C]acetyl-coenzyme A was diluted with unlabeled acetyl-coenzyme A (Sigma Chemical Co.) to give a final concentration of 0.2 mM. The mixture was incubated for 12 minutes at 37°C, and the reaction was stopped by the addition of 5 ml of ice-cold 10 mM sodium phosphate buffer (pH 7.4). One milliliter of 3-heptanone containing 15 mg sodium tetraphenyl boron (Sigma Chemical Co.) was added to the tubes and the contents were shaken lightly for 1 minute. After centrifugation at 3000 rpm for 5 minutes, 0.5 ml of the organic phase containing the [14C]acetylcholine synthesized was transferred into vials containing 10 ml of 3a70B scintillation cocktail (Research Products International Corp.). The radioactivity was determined in the liquid-scintillation counter (LS 8000, Beckman Instruments, Inc.) at a counting efficiency of 89%. Blank tubes with no tissue added contained less than 0.1% of the added radioactivity and were subtracted from the sample results.

**Norepinephrine Content**

Norepinephrine was analyzed by high pressure liquid chromatography using an electrochemical detector, according to the method of Felice et al. (1978).

**In Vitro Studies**

Ring segments (4 mm long) of the blood vessels were cannulated with a stainless steel rod of hemispherical section and a short piece of platinum wire and mounted in an isolated tissue bath that contained 30 ml of Krebs’ bicarbonate solution at room temperature. The platinum wire was bent into an inverted U shape and anchored to a plastic gate which could be moved up and down by a fine control micrometer. The steel rod was connected to a Statham strain gauge (G10b, 0.15 oz) for isometric recording of changes in force. This method has been described previously in detail (Bevan and Osher, 1972; Lee et al., 1976). After 5–10 minutes, the temperature of the Krebs’ bicarbonate solution was increased gradually to and maintained at 37°C. Resting tension then was adjusted to optimum length and a period of 1 hour was allowed for equilibration. For studies on the response to drugs, both agonists and antagonists were added directly to the organ bath.

After equilibration, a contractile response to an approximate ED50 concentration of norepinephrine (NE) (10−7 to 10−5 M), the precise level depending on the segment, was elicited. After being washed, the tissue was incubated in guanethidine (5 x 10−6 M) for 30 minutes to inactivate the sympathetic innervation, as confirmed in each experiment by the absence of a contractile response to electrical transmural nerve stimulation (TNS) in the absence of muscle tone. Guanethidine was maintained throughout the experiment. After equilibration, tone was induced by prostaglandin (PGF2α 5 x 10−8 M), TNS was applied as 20-second trains of 0.3-msec pulses at 4 Hz at a voltage just below that which in the presence of tetrodotoxin (3 x 10−7 M) caused a just detectable dilator response using a device that offers a low source impedance to the stimulating electrodes (Duckles and Silverman, 1980). When testing for pharmacological blockade with atropine, control TNS responses were elicited in the presence of PGF2α-induced tone. After the tissue was washed, atropine was added, and 15–20 minutes later, tone was reestablished with the prostaglandin and the periods of TNS repeated. In many studies, an adjacent control tissue segment was treated in an identical manner except that no atropine was added to the tissue bath. The peak dilation was expressed as a percentage of the level of active tone and/or grams tension decrease.

**Results**

**Choline-Acetyltransferase Levels**

The levels of ChAT in various arterial and venous segments studied are shown in Table 1 and illustrated diagrammatically in Figure 1. Values are low in the supply arteries to the head, the vertebral and at least the proximal part of the common carotid. These levels are not significantly different from those found in the radial and subclavian arteries chosen as representative of systemic vessels from the arm and chest and probably are not functionally significant (see below). Varying levels of ChAT in excess of these were found in all segments of the cranial arterial tree, both cerebral and extracerebral, that were examined. They were particularly high in the pial arteries, both cerebral and cerebellar, the arteries to the salivary glands, to the tongue—in particular, the distal part of the lingual and to the nose, especially the sphenopalatine artery. Intermediate levels were found in the external and internal maxillary arteries, including the rete. Activities measured in the posterior auricular, infraorbital, distal facial, and distal common carotid arteries were significantly higher than those in the proximal carotid and vertebral arteries. Enzyme levels in the lingual vein and other representative veins from the chest and forelimb were of the same order of magnitude as in the common carotid artery, and were probably not functionally significant.

In order to determine whether the activity assayed was entirely attributable to ChAT, tissue enzyme levels in adjacent segments were measured in the presence of (2-benzoylethyl) trimethylammonium choline (BETA), a stable and selective inhibitor of ChAT (Rowell et al., 1978). The BETA ID50 values for...
the inhibition of cat iris ChAT were $6.5 \times 10^{-6}$ M and 90% inhibition of activity was achieved at approximately $2.5 \times 10^{-3}$ M. Also shown in Table 1 are some activity values determined in the presence of BETA ($10^{-3}$ M). It is clear that the BETA-resistant activity is small in proportion to total values in arteries with high ChAT activity and can, from the point of view of this study, be disregarded. However, when arterial wall levels of this enzyme are low, the BETA-insensitive component is a significant proportion of the total. In the case of the proximal common carotid artery for example, it is 27%.

### Norepinephrine Content

Norepinephrine content values for selected arteries are included in Table 1 and in Figure 2 shown plotted against corresponding ChAT determinations. The correlation between the two groups of data is not significant ($r = 0.133$). There is less variation in the NE compared with the ChAT values in the series of cat vessels studied; the high levels of ChAT found in cerebral and lingual arteries are not associated with corresponding high catecholamine content.

### Neurogenic Dilation

The neurogenic dilator response was taken as that to a 20-second train of short pulses at 4 Hz delivered at a voltage just below that which "breaks" through pretreatment with TTX ($3 \times 10^{-7}$ M). Since the level of tone at the time of TNS was not the same in all preparations, and varied in the same preparation with time, peak relaxation was expressed as a percentage of the level of active tension at the time of TNS was not the same in all preparations. Such vessels still relaxed to papaverine and nitrites. Thus, there was a clear relation between the presence of higher levels of ChAT and the occurrence of neurogenic dilation (Fig. 3).

The characteristics of the neurogenic dilation were independent of the pharmacological agent used to initiate tone. PGF$_{2}$, was utilized as it initiated tone more consistently, and for a longer period of time,

### Table 1

<table>
<thead>
<tr>
<th>Blood vessel</th>
<th>ChAT (nMACH/g wet wt per hr)</th>
<th>BETA ($10^{-3}$ M)</th>
<th>NE content (μg/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid artery Prox 1/3</td>
<td>13.4 ± 2.8 (10)</td>
<td>3.6 ± 0.4 (4)</td>
<td>3.55 ± 0.55</td>
</tr>
<tr>
<td>Dist 1/3</td>
<td>19.6 ± 3.6 (9)</td>
<td>6.5 ± 0.8 (4)</td>
<td>2.50 ± 0.26</td>
</tr>
<tr>
<td>Lingual artery Prox</td>
<td>80.7 ± 20.5 (6)</td>
<td>14.6 ± 5.2 (5)</td>
<td>13.41 ± 0.83</td>
</tr>
<tr>
<td>Dist</td>
<td>245.7 ± 43.5 (8)</td>
<td>50.2 ± 9.9 (6)</td>
<td>14.79 ± 0.82</td>
</tr>
<tr>
<td>Facial artery Prox 1/3</td>
<td>147.1 ± 20.1 (5)</td>
<td></td>
<td>2.03 ± 0.43</td>
</tr>
<tr>
<td>Dist 1/3</td>
<td>30.5 ± 4.5 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal maxillary artery</td>
<td>134.5 ± 11.5 (4)</td>
<td></td>
<td>3.29 ± 0.26</td>
</tr>
<tr>
<td>Prox to rete</td>
<td>68.7 ± 18.0 (5)</td>
<td>12.5 ± 3.5 (4)</td>
<td></td>
</tr>
<tr>
<td>Dist to rete</td>
<td>85.0 ± 19.8 (5)</td>
<td>15.0 ± 3.5 (3)</td>
<td></td>
</tr>
<tr>
<td>rete</td>
<td>135.3 ± 11.5 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphenopalatine artery</td>
<td>84.5 ± 24.8 (5)</td>
<td>13.2 ± 4.8 (3)</td>
<td></td>
</tr>
<tr>
<td>Infraorbital artery</td>
<td>50.5 ± 11.8 (5)</td>
<td>15.9 ± 8.4 (5)</td>
<td></td>
</tr>
<tr>
<td>Superficial temporal A.</td>
<td>22.2 ± 2.6 (19)</td>
<td>3.2 ± 1.9 (6)</td>
<td>6.2 ± 0.45</td>
</tr>
<tr>
<td>Post. auricular artery</td>
<td>47.0 ± 5.8 (5)</td>
<td></td>
<td>13.50 ± 1.053</td>
</tr>
<tr>
<td>Br. to parotid gland</td>
<td>212.0 ± 42.7 (3)</td>
<td></td>
<td>5.89 ± 1.2</td>
</tr>
<tr>
<td>Br. to sublingual gland</td>
<td>193.6 ± 63.1 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertebral artery</td>
<td></td>
<td>1.47 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>Pre-canal</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canal</td>
<td>6.2 ± 4.0 (3)</td>
<td></td>
<td></td>
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<tr>
<td>Atlas</td>
<td>10.8 ± 1.8 (3)</td>
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<tr>
<td>Basilar artery</td>
<td>142.3 ± 20.6 (8)</td>
<td>2.85 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>Mid cerebral artery</td>
<td>116.0 ± 42.5 (4)</td>
<td>2.66 ± 0.67</td>
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<tr>
<td>Post cerebral artery</td>
<td>234.2 ± 45.6 (4)</td>
<td>3.50 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>Post inf. cerebellar artery</td>
<td>152.6 ± 28.7 (3)</td>
<td>3.07 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Radial artery</td>
<td>10.42 ± 3.5 (4)</td>
<td>3.70 ± 2.02 (4)</td>
<td>3.47 ± 0.46</td>
</tr>
<tr>
<td>Subclavian artery</td>
<td>7.5 ± 2.0 (8)</td>
<td>2.06 ± 1.11 (4)</td>
<td>1.19 ± 0.30</td>
</tr>
<tr>
<td>Cephalic vein</td>
<td>14.2 ± 3.2 (8)</td>
<td>5.1 ± 3.6 (4)</td>
<td></td>
</tr>
<tr>
<td>Lingual vein</td>
<td>10.8 ± 2.5 (12)</td>
<td>4.8 ± 0.9 (6)</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± st. (n)
than other vasoconstrictors, such as serotonin, nor-
epinephrine, and vasopressin.

Effect of Atropine

The effect of atropine (5 × 10⁻⁷ M) on the neuro-
ogenic dilation in some of the arteries with high ChAT
content, specifically the basilar, middle cerebral, lingual, and internal maxillary, was determined. At 4
Hz, exposure to atropine caused a significant reduc-
tion in the magnitude of the dilator response of all
vessels. The results are shown in Table 2 and illus-
trated in Figure 4. There was considerable variation in
the pattern of the dilator effect and of the conse-
quence of exposure to atropine. The tracings chosen for Figure 4 illustrate some of this variation. This dose
of atropine had no effect on the dilation of the lingual
and basilar arteries to papaverine (10⁻⁷ and 10⁻⁸ M)
and sodium nitrite (3 × 10⁻⁴ and 3 × 10⁻³ M). These
doses cause relaxation that spans the neurogenic di-
lation in these vessels when stimulated at 4 Hz. The
mean dilations after atropine expressed as a percent
of that before, for these four responses of the basilar
artery, were: 95 ± 4.5, 97 ± 8.3, 101 ± 6.7, 94 ± 6.2,
respectively (in each case n = 6).

Discussion

ChAT activity was considerably higher in all arte-
rial segments taken from the cat cranial tree than in
the two pairs of arteries that supply the head, the
common carotid, and vertebral and several represent-
atives of the systemic circulation, the subclavian and
radial arteries. Little activity was found in veins, even

<table>
<thead>
<tr>
<th>Artery</th>
<th>Control</th>
<th>Atropine</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basilar</td>
<td>33.60 ± 8.90 (4)</td>
<td>10.55 ± 7.9 (4)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Internal maxillary</td>
<td>30.81 ± 9.10 (6)</td>
<td>7.40 ± 6.23 (6)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Lingual</td>
<td>49.42 ± 4.66 (17)</td>
<td>35.14 ± 4.7 (17)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Middle cerebral</td>
<td>29.01 ± 3.43 (6)</td>
<td>7.48 ± 5.33 (6)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Dilator responses are expressed as percent relaxation. Stimulation trains consisted of 80 pulses of 0.3 msec at 4 Hz using
maximum TTX (3 × 10⁻⁷ M)-sensitive voltage. Stimulation was
applied in the presence of PGO (5 × 10⁻⁴ M) and guanethidine (5 × 10⁻⁷ M).

Table 2: Effect of Atropine (5 × 10⁻⁷ M) on the Neurogenic Dilator Responses of Some Cat Cranial Arterial Segments to Electrical Stimulation of Their Intramural Nerves
FIGURE 4. Effect of atropine (5 × 10^{-7} M) on neurogenic dilation of artery segments to transmural nerve stimulation. Stimulation trains of 80 pulses of 0.3-msec duration at 4 Hz using maximum TTX (3 × 10^{-7} M)-sensitive voltages in the presence of PGF_{2α} and guanethidine (5 × 10^{-6} M) were employed.

those such as the lingual, that were associated with arteries with high enzyme levels.

Highest levels of ChAT are found in the pial arteries and those to salivary glands, tongue, and, to a lesser extent, to the nose. Significant levels of ChAT have been previously determined in small pial vessels and microvessels of the rat (Hardebo et al., 1977). These are sites where, with the possible exception of brain microvessels, neurogenic dilation has been established or at least strongly suggested by in vivo investigations (see introductory paragraphs).

ChAT activity could reflect the presence of efferent neurones, especially their terminations and, possibly, synapses in the walls of the segments from these arteries of neurones that supply the more distal arborizations of the vascular beds. However, perivascular ramification is not the only route; for example, additional fibers to the nasal mucous membrane vessels presumably also would pass via the sphenopalatine ganglion and the nerve of the pterygoid canal (Anggard, 1974). The significant levels in the external and internal maxillary arteries may well reflect in part fibers passing to more distal sites originating from cranial nerves VII and IX. The intermediate to low activity encountered in some of the branches of these arteries may represent fibers innervating the vessels themselves, but also passing to the blood supply to skin and local hair follicles and sweat glands.

In view of the effect of BETA, which at ID_{50} values, albeit 10^{-3} M, reduced the measured activity in the common carotid artery from 13.4 to 3.6 units, there is some uncertainty concerning the interpretation of low levels measured in the ChAT assay. The common carotid artery and other vessels where similar enzyme levels were found invariably did not dilate to transmural nerve stimulation provided TTX-sensitive stimulation parameters were used. Such levels of ChAT may represent other acetylated compounds, such as acetylcarnitine. Some cholinergic features are found associated with sympathetic nerves and other primarily noncholinergic structures (Eranko et al., 1970; Hume and Waterson, 1978). This may possibly reflect their origin from neural crest cells which are considered pluri-potential for cholinergic and adrenergic neurones early in development (Le Lievre et al., 1980).

However, this study shows that neurogenic dilation is found experimentally only in those vessels with ChAT activity raised above the minimum levels: about 20–30 units found in a number of systemic vessels. ChAT has been reported in a number of tissues devoid of innervation, the human placenta (Morris 1966) and sperm (Harbison et al. 1974), for example. Its role in these sites is not known. However the relationships found in this study between ChAT and neurogenic dilation emphasizes its primarily neural role in the cranial vasculature. No systematic determination of ChAT levels in the vasculature has been previously made. Determinations of cholinesterase in blood vessels by histochemical methods have been published, but there are serious and genuine reservations regarding their functional significance. The presence of acetylcholinesterase is not necessarily indicative of cholinergic neurons (Korrle, 1963); in fact, this enzyme has been seen in association with seemingly typical sympathetic neurons (Hume and Waterson, 1978). However, the observation that the density of enzymes determined histochemically does not alter after chronic sympathectomy (Edvinnson et al., 1976; Lee et al., 1978) is somewhat compelling with regard to its significance in cerebral vessels. The distribution of ChAT is similar to acetylcholine in cholinergic systems (Yamamura and Snyder, 1973), and its pattern parallels high affinity choline uptake in cerebral and one extracerebral artery of three species (Florence and Bevan, 1979). This uptake system is believed to be rate limiting in acetylcholine synthesis (Jope, 1979). Despite these arguments for a functional role of the enzyme, its significance and the possible contribution of a cholinergic transmitter to the transsynaptic mechanism in these vessels remains a matter of debate.

The magnitude of the neurogenic dilation but not that to nitrite and papaverine was significantly reduced by atropine (5 × 10^{-7} M) in segments from four of the arteries that exhibited the higher ChAT activity. This finding contrasts with our earlier conclusion (Lee et al., 1978) that neurogenic dilation in cerebral arteries is unaffected by atropine. We can only conjecture concerning this difference. The doses of antagonists are essentially the same, and our results suggest that the atropine reduction is specific. Our current small vessel myograph allows a vessel of <0.5 mm o.d. to
be set up more easily than the model in use for the
studies reported in 1978. In addition, we have now
used a modified electrical stimulation unit (Duckles
and Silverman, 1980) that provides better stimulus
control and regulation. In addition, we now adjust
the stimulation voltage in the presence of TTX in a large
percentage of tissues at the beginning of the planned
experiment and then continue using this voltage after
TTX washout. In the earlier study we relied more
frequently on a voltage predetermined before the
series was started. Other conditions are, as far as we
are aware, identical. We are inclined to give more
weight to the positive findings with atropine, espe-
cially in view of the increasing evidence in support of
a cholinergic mechanism in cerebral and other cranial
vessels (Florence and Bevan, 1979; Bevan et al, 1981).

The data presented in this paper may be interpreted
as defining a functional dilator system associated with
ChAT confined to certain tissues of the head. This
does not imply that other dilator outflows do not exist
(Bell, 1975; Su and Lee, 1975), but that this system is
separate from those. This study also emphasizes that
this system can be distinguished from the sympathetic
adrenergic outflow, a conclusion reached by Lee et al.
(1978) who found that neurogenic dilation remained
unchanged in magnitude after chronic sympathe-
tomy by bilateral superior cervical ganglionectomy.

Recently, Lundberg et al. (1979), in a provocative
paper, commented on the occurrence of neurones in
which both acetylcholinesterase and vasoactive intes-
tinal polypeptide (VIP) were present in autonomic
ganglia, certain nerves, and in nerve fibers around
sweat glands and certain blood vessels. There is evi-
dence that VIP is found in several of the arteries and
their tributaries included in this study [cerebral (Lar-
sen et al., 1976), nasal (Uddman et al., 1978), and
salivary gland (Bloom and Edwards, 1980)] which are
rich in ChAT. We have found that VIP is a potent
vasodilator in these vessels and evidence for a similar
association (Bevan et al., 1981). However, the signifi-
cance of this association remains to be defined.

During embryonic development, an invagination of
the ectoderm—the outer of the three primitive cell
layers—forms part of the primitive foregut. The junc-
tion between this ectoderm and the endoderm re-
sponsible in the adult for the lining of the alimentary
canal occurs in man at any rate at the fauces—at the
back of the mouth. The mucous membrane of the
tongue, and the secreting cells of the salivary
glands are derived from this ectodermal inpushing
(Arey, 1974). The central nervous system also origi-
nates from the infolding of this primitive outer cell
layer. Thus, in a developmental and embryonic sense,
those tissues whose supplying arteries have the high-
est levels of ChAT have a similar developmental
origin. ChAT levels in blood vessels to other ectoder-
mal-derived tissues—sweat glands and hair follicles—
have not been measured. The circumstantial evidence
of this study, however, is consistent with such an
innervation passing via the branches of the external
and possibly internal maxillary artery.

With respect to the functional significance of this
system, any attempt at a hypothesis that encompasses
dilator neural systems to such diverse tissues can only
be speculative (Bevan et al., 1981). During thermal
stress, in order to provide fluid to the nose, mouth,
and salivary glands for evaporative cooling, there is
a remarkable dilation in these tissues. It is possible
that, since there are many connections between cere-
bral and extracerebral blood vessels, the flow of blood
to the brain might be jeopardized by such extensive
vasodilation. Concomitant cerebral vasodilation might
minimize this possibility. Thus it seems likely that
neurogenic dilation of nose, mouth, and pharynx,
together with, brain, might be part of a general
homeostatic mechanism to help maintain the con-
stancy of brain temperature.

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Address for reprints: Dr. J.A. Bevan, Department of Pharmacol-
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