Histochemical Study on the Innervation of Noradrenergic and Acetylcholinesterase-Positive Nerves in the Internal Carotid Artery and Cerebral Arterial Tree of the Duck

Kōichi Andō¹, Noboru Fujihara² and Haruo Kusaba³

¹ Biological Laboratory, Department of Industrial Chemistry, Faculty of Engineering, Kyushu Sangyo University, Matsukadai, Higashi-ku, Fukuoka 813-8530, Japan.
² Laboratory of Animal Reproduction, Faculty of Agriculture, Kyushu University, Fukuoka 812-8251, Japan.
³ Muromi Animal Hospital, Muromi, 1-11-9, Sawara-ku, Fukuoka 814-0015, Japan.

The pattern of noradrenergic (NA) and acetylcholinesterase-positive (AChE) innervation in the internal carotid artery (ICA) and cerebral arterial tree was investigated in the duck. Cerebral perivascular NA and AChE nerves mainly enter the cranial cavity along the ICA. The NA nerves via the ICA originate exclusively in the axons within the sympathetic internal carotid nerve (SICN), while AChE nerves from the same vascular route have their major origin at the AChE nerve cells that are contained in the stem nerve bundle accompanying the SICN. In addition, a portion of AChE nerves in the internal carotid system (ICS) is derived from AChE nerve cells intrinsic to this cerebral arterial system. The major arteries of the duck ICS, as well as its ICA, were innervated by abundant NA and AChE nerves with approximately the same density. This finding is quite different from an unbalanced NA and AChE innervation in the corresponding arteries of the quail that is characterized by a markedly lesser density or lack of AChE nerves, suggesting that there is significant species difference in the cholinergic mechanisms for functioning of these arteries in the blood supply to the avian brain.


Key words: noradrenergic nerves, acetylcholinesterase-positive nerves, internal carotid artery, cerebral arterial system, duck.

Introduction

The cerebrovascular bed in mammals is known to be multiply innervated by noradrenergic (NA), acetylcholinesterase-positive (AChE) nerves (Wasano, 1979), various types of peptidergic nerves (Uddman and Edvinsson, 1989), and nitric oxide-synthesizing nerves (Nozaki et al., 1993). The roles of these neurochemicals as a direct vasomotor (constrictor or dilator), neuromodulator and trophic factor of cerebral circulation have been well documented pharmacologically (Owman et al., 1986; Edvinsson, 1991; Faraci and Brian, 1994).

Angioarchitecture of the internal carotid artery (ICA) and cerebral arterial system in avian species, as being represented by the formation of internal carotid anastomosis (ICAS) near the cranial cavity, and the unilateral communication between the internal carotid and vertebrobasilar systems (ICS, VBS), is greatly modified from that of
mammals and other vertebrates (Baumel and Gerchman, 1968; Jones and Johansen, 1972; Ando et al., 1996). Thus, it is an interesting subject to investigate, from a standpoint of comparative neurology, as to which neurogenic mechanisms are operated in the avian cerebrovascular system. Our previous study revealed that the ICA and major cerebral arteries in the quail received a rich supply of NA nerves, but had a markedly less density of or no AChE nerves (Ando et al., 1996). This innervation pattern is divergent from the general mammalian pattern that the major cerebral arteries are densely invested with NA and AChE nerves, despite very poor supply of both nerve types in the ICA (Wasano, 1979). To determine whether the pattern of NA and AChE innervation found in the quail is regarded as a common feature for the avian cerebrovascular innervation, the present study was undertaken in the duck.

**Materials and Methods**

**Tissue preparation**

Twelve adult ducks (Anas platyrynchos domesticus) of both sexes were used in this study. The birds were bilaterally perfused through the right and left common carotid arteries with cold Ringer’s solution under ethyl ether anesthesia. The brain and the internal carotid artery (ICA) were removed either immediately, or after perfusion with 4% buffered formaldehyde (FA). Fixed materials were subsequently postfixed in the same fixative for 1 h at 4°C. For wholemount preparations, the cerebral arterial tree was carefully dissected out from the brain, and the ICA was stripped of its outer adventitial connective tissue. Similarly, nerve bundles attendant to the ICS were dissected and carefully stripped of their connective tissue. For sectioning, small segments of the ICS containing the nerve bundles were quickly frozen in isopentane chilled with dry–ice.

**Formaldehyde fluorescence and AChE histochemistry**

For demonstration of aminergic neurons, unfixed wholemount materials were stretched over non-fluorescent glass slides. They were air-dried, then treated with formaldehyde gas evaporated from paraformaldehyde (relative humidity = 47%) for 1 h at 80°C. For demonstration of AChE-positive neurons, fixed whole-mounts and cryostat sections (15 μm in thickness) were maintained in Karnovsky’s medium without acetyl choline iodide (Sigma) for 30 min at 4°C, and then incubated in the complete medium containing 2 × 10^{-4} tetraisopropylpyrophosphramide (Sigma) as an inhibitor of non–specific cholinesterase activity for 1 h at 20°C. The detailed procedures of the formaldehyde histofluorescence and AChE staining have already been described by Ando (1981).

**Results**

**Aminergic innervation**

The sympathetic internal carotid nerve (SICN), which emanated from the superior cervical ganglion (SCG) and emitted a bright FA fluorescence, subdivided further to build up dense plexuses of NA nerves throughout the ICA. The density of NA nerve plexuses became much high in the distal direction, particularly around the ICAS,
consisted of fine and complicated meshworks of thin axons (Fig. 1). The majority of NA fiber bundles from the SICN entered the cranial cavity through the cerebral carotid artery (CCA), the intraranial part of the ICA, and spread widely over the cerebral arterial tree. The presence of such fluorescent fiber bundles was also seen to run along the cerebroethmoidal artery (CEA) (Fig. 2).

The supply of NA nerves to the cerebral arterial tree was distinctly high in the ICS than in the VBS. It was particularly rich over the walls from the distal part of the anterior ramus (AR) to the middle cerebral artery (MCA), formed complicated meshworks which were organized mainly from longitudinally-oriented thin fibers (Fig. 3). In the posterior and anterior cerebral arteries (PCA, ACA) and the CEA, the meshworks became coarser and more elongated, but were considerably well-developed. The posterior ramus (PR) received a relatively rich or moderate number of NA nerves. However, the density of nerves decreased markedly in the basilar and vertebral arteries (BA, VA), and a few fluorescent axons were scattered spirally or in parallel to these vascular axes (Fig. 4). There were no ganglionic structures with FA fluorescence along the ICA and the major cerebral arteries at all parts of the brain.

**AChE–positive innervation**

The SICN comprised a considerable amount of AChE axons mainly around its outer part (Fig. 5). As shown in Fig. 6, another stem nerve bundle, of which AChE-
Figs. 5-12. Photomicrographs of section (5) and whole-mounts (6-12) with acetylcholinesterase (AChE) staining in the internal carotid artery (ICA), sympathetic internal carotid nerve (SICN) and the major cerebral arteries. Fig. 5. The middle part of the ICA (asterisk) and the sympathetic internal carotid nerve (SICN) accompanying it. Note abundant AChE nerves at the medio-adventitial border of the ICA (arrows). Fig. 6a and b. AChE stem nerve bundle (arrows) accompanying the SICN. Arrowhead indicates a small group of AChE ganglion cells (a) and its high magnification (b). Fig. 7. The distal part of the ICA. Fig. 8. The cerebral carotid artery (CCA), anterior ramus (AR), and the posterior ramus (PR). Note many AChE fiber bundles projecting to the ICS via the CCA. Fig. 9. The cerebroethmoidal artery. Arrow indicates AChE fiber bundles. Fig. 10a and b. The proximal to middle parts of the AR. Arrow indicates two to three contiguous AChE nerve cells (a) and its high magnification (b). Fig. 11. The middle cerebral artery. Fig. 12. The basilar and posterior inferior cerebellar arteries (BA, PICA). Arrows indicate nerve fibers with a weak AChE activity. Figs. 5, 6a-9 and 10a-12, \( \times 83 \); Figs. 6b and 10b, \( \times 250 \).
activity was distinctly much high as compared to the SICN, ran in close association to the SICN. This AChE stem nerve bundle gave off many fiber bundles in its course, and contained many AChE cell bodies that were small to medium-sized, with diameter of 25–35μm, and situated singly or in small group. AChE axons arising from these fiber bundles were densely distributed along the entire length of the ICA, formed particularly well-developed meshworks in the distal ICA including the ICAS (Figs. 5, 7). AChE fiber bundles near the cranial cavity projected mainly to the cerebral arterial tree via the CCA (Fig. 8), ran rostrally along the anterior ramus (AR), and provided abundant AChE axons to all the major arteries of the ICS. A few AChE fiber bundles were also detected in the walls of the IEA (Fig. 9). Their axons could usually be traced to the upper AR just before bifurcating into the CEA and MCA, but did not reach as far as the middle AR. In addition, a small number of AChE nerve cells, of which the diameter was about 23μm, were scattered alone or in small cluster in the walls of the major cerebral arteries of the ICS, mainly of the AR (Fig. 10).

The density of AChE nerves supplying the ICS were particularly high in the walls from the upper AR to the MCA (Fig. 11). Here, an abundant number of thin fibers, which were derived from AChE fiber bundles via the ICA and CEA, made up longitudinally-arranged meshworks that were comparable in density to those in the distal ICA. The plexuses of AChE nerves in the CEA were also considerably well developed (Fig. 9). The middle to proximal AR had a relatively rich supply of AChE nerves (Fig. 10). A moderate number of nerves were observed in the walls of the ACA and PCA. In the VBS, the innervation density became markedly lowered. Although a moderate number of AChE nerves, which arose from the fiber bundles on the CCA, were consistently found along the PR, the BA and VA were supplied with only a few nerves stained weakly for AChE, or sometimes had no nerves with this enzyme activity (Fig. 12).

**Discussion**

In our previous study, we noted that the ICA and major cerebral arteries of the quail were richly supplied with abundant NA nerves, but had a markedly poor or no supply of AChE nerves (ANDO, et al., 1996). In the present study on the duck, we have shown the rich innervation of the corresponding arteries by NA and AChE nerves with about equal density. Thus, clear species difference was observed with regard to the density of AChE nerves innervating the ICA and cerebral arterial system of these two birds. Each pattern of NA and AChE innervations found in the ICA of the two avian species is unusual when compared with the innervation pattern for mammals that is characterized by a very poor supply of both nerve types (WASANO, 1979). On the other hand, the supply of NA and AChE nerves to the duck major cerebral arteries is distinctly much high in the ICS than in the VBS, showing a tendency similar to the cerebrovascular innervation in mammals (WASANO, 1979).

Cerebral perivascular NA nerves in the duck enter the cranial cavity along the ICA, IEA, and possibly VA. The majority of them originates in the SICN that emanates from the SCG, and comes from the ICA. This finding is in good agreement
with the major source and pathway of cerebrovascular NA innervation reported for mammals (Ando et al., 1991) and quail (Ando et al., 1996).

In rat, there is conclusive evidence that the principal origin for cerebrovascular AChE nerves is the sphenopalatine ganglion (Harada and Weir, 1986; Suzuki et al., 1988; Edvinsson et al., 1989). The axons from here pass through the CEA as fiber bundles, and distribute widely over the major arteries more rostral than the middle BA. In addition, a few axons from another two facial or glossopharyngeal parasympathetic ganglia, the internal carotid mini-ganglion (ICMG) and the otic ganglion, project to the caudal half of the ICS via the CCA. In the duck, nearly all of the cerebral perivascular AChE nerves come also from the CEA and CCA as fiber bundles. However, AChE neuronal projections from these two vascular routes are in contrast to that of the rat: nerves via the CCA extend to both the ICS and VBS, and provide abundant axons to all the major arteries of the ICS, whereas nerves via the CEA are distinctly less numerous than those via the CCA, and do not reach as far as the middle AR caudally. Since AChE fiber bundles on the CCA arise directly from the stem nerves bundle accompanying the SICN, it is highly probable that cerebral perivascular AChE nerves in the duck have their major source at the nerve cells that are contained in this stem nerve bundle. These extracranial AChE neurons do not express FA fluorescence specific for catecholamine, and are hence considered to be parasympathetic in nature, probably homologous to the ICMG in mammals (Vasquez and Purves, 1979; Gibbins et al., 1984; Hardebo et al., 1991). The present study has further shown the localization of a few AChE nerve cells in the duck ICS, mainly on wall of the AR. These intrinsic AChE neurons are also negative for FA fluorescence. Accordingly, they, at least on the AR, are interpreted as being the intracranial outflow from parasympathetic AChE neuronal tract via the CCA. The presence of AChE nerve cells in the cerebral arterial system has also been ascertained from the quail (Ando et al., 1996), but has not been demonstrated in any vertebrate species other than the bird.

Which neurogenic controlling mechanisms are involved in the avian cerebral circulation have not yet been elucidated. However, pharmacological effect of NA as a direct vasoconstrictor (Edvinsson and Owman, 1974) and a trophic factor (Bevan, 1984), and that of ACh as an indirect vasodilator (Furchgott and Zawadski, 1980) and an inhibitor for release of NA from sympathetic nerves (Duckles and Kennedy, 1982) have been well established from the mammalian cerebral vascular system. Therefore, the rich NA and AChE innervation of the duck ICA and ICS, particularly of the distal ICA, upper AR and MCA, must be considered in relation to the critical role of adrenergic and cholinergic vasomotor actions for functioning of these arterial regions in the blood supply to the brain in this bird. Further, the distinct difference in the innervation density of AChE nerves in the ICA and cerebral arterial system of the duck and quail may indicate the possibility that there is significant species difference in the parasympathetic cholinergic mechanisms responsible for the regulation of avian cerebral circulation.
References


アヒル内頸動脈，脳動脈系におけるノルアドレナリン含有およびアセチルコリンエステラーゼ陽性神経支配に関する組織化学的研究

安藤光一1）・藤原_AndO2)・草場治雄3)

1）九州産業大学工学部，福岡市 813-8530
2）九州大学農学部，福岡市 812-8251
3）室見動物病院，福岡市 814-0015

アヒル内頸動脈，脳動脈におけるノルアドレナリン含有（NA）およびアセチルコリンエステラーゼ陽性（AChE）神経の分布・密度について，組織化学的に調べた。本鳥類の内頸動脈および脳動脈には，これら２神経の類似した支配様相が観察された。NAおよびAChE神経は内頸動脈の全域にわたり細密な網状網を形成し，内頸動脈吻合部付近で著しく高密度であった。脳動脈系では，NAおよびAChE神経は椎骨・脳底動脈系よりも内頸動脈に沿って明らかな優勢分布を示し，支配密度は前幹枝遠部から中大動脈にかけて特に高かった。脳血管NAおよびAChE神経は主に内頸動脈経由で脳動脈系へ投射するが，NA神経は上頸神経節から放出された内頸交感神経幹（SICN）に専ら由来する。他方，AChE神経はSICNに伴行する神経幹内のAChE神経細胞に起因する可能性が高い。これに加えて，内頸動脈に分布するAChE神経の一群は脳動脈系に存在するAChE神経細胞から発するものと考えられる。結論として，今回報告したアヒル内頸動脈，脳動脈のNAおよびAChE神経の支配様相は前回報告したAChE神経の著しい低密度あるいは欠如により特徴づけられるウズラの不均衡なNAおよびAChE神経支配を大きく異なる。このことは鳥類の脳循環系に関与しているコリン作動神経調節機構に明らかに種間差があることを示唆しているものと考える。

（家禽会誌，35：220～227，1998）

キーワード：NA神経，内頸動脈，脳動脈，アヒル