Monoamine Oxidase in the Hypothalamo-hypophysial Region of the Brown Smooth Dogfish, *Triakis scyllia*

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**Synopsis**

Distribution of monoamine oxidase (MAO) was histochemically determined in the hypothalamo-hypophysial region of the dogfish, *Triakis scyllia*. MAO positive neurons, which seemed to be monoaminergic, were observed in the nucleus medius hypothalamicus (NMH) and the nucleus tuberis (NT). NMH was mainly innervated by the MAO-positive fibers arising from the tractus strio-thalamicus et hypothalamicus, and NT was innervated by those from the commissure pre-infundibulare.

The median eminence was innervated by MAO-positive fibers from both NMH and NT, and these were more abundant in the posterior region. These fibers tended to accumulate around the blood capillaries of the primary plexus. The possibility of the involvement of a monoaminergic mechanism in controlling the secretion of adenohypophysial hormones is discussed.

In the neurointermediate lobe, MAO-positive fibers were often observed between the cells of this lobe. The cylindrical cells around the sinusoid-like capillaries showed rather moderate MAO activity. The cells in the cellular cord of each lobule showed very weak activity. MAO positive cells were also observed in the pars distalis.

The presence of catecholamines in the avian and mammalian median eminence has been suggested by the electron microscopic identification of small electron dense granules (see Kobayashi, 1964; Kobayashi and Matsui, 1969). At about the same time, catecholamines were found by fluorescence microscopy in the superficial zone of the avian and mammalian median eminence (Fuxe, 1964; Björklund et al., 1968; Sharp and Follett, 1968; Fuxe and Hökfelt, 1969). Furthermore, monoamine oxidase (MAO) has been demonstrated in the same region of the avian and mammalian median eminence that contains catecholamines and small electron dense granules (Matsui and Kobayashi, 1965; Follett et al., 1966; Urano, 1968). All these observations mentioned above leave no doubt that a monoaminergic mechanism is functioning in the median eminence of these animals.

In this investigation, the distribution of MAO activity in the hypothalamo-hypophysial region of the elasmobranch was determined. Special attention was directed to the median eminence in order to examine whether or not a monoaminergic mechanism functions in this region of the elasmobranch as it does in higher vertebrates.

**Material and Method**

Seven brown smooth dogfish (*Triakis scyllia*), ranging from 79 to 97 cm in total length, were captured near the Misaki Marine Biological Station, Kanagawa Prefecture and they were maintained several months in a sea water pool before killing in November and December.

For the demonstration of MAO, the tryptamine-
tetrazolium method (Glenner et al., 1957) was used with some modifications (Urano, 1968). For sectioning, the hypothalamo-hypophysial region was cut out upon decapitation. The tissue was frozen in isopentane maintained at $-86^\circ C$ through use of acetone and dry ice. Fresh frozen sections at 20 $\mu$ were prepared on a cryostat and mounted on cover slips. Immediately after being taken out of the cryostat, the sections were dried with a hair-drier at room temperature. Then the cover slips with the tissues were incubated for 40 minutes in the following freshly prepared reaction mixture at 37$^\circ C$. The reaction mixture consisted of 5 mg tetranitro-blue tetrazolium, 25 mg tryptamine-HCl, sodium sulfate anhydrous (1.67 g), 0.1 M phosphate buffer (5.0 ml, pH 7.6) and deionized water (15 ml).

Specificity of the MAO reaction was confirmed on the tissues of the hypothalamo-hypophysial region by the following tests: (1) incubation of sections exposed to 100$^\circ$C water vapour for 1 min, (2) incubation of sections in the reaction mixture without substrate, (3) incubation of sections pretreated with MAO inhibitor (10$^{-3}$M or 10$^{-4}$M p-phenyl-isopropylhydrazine, or 10$^{-3}$M or 10$^{-4}$M tranylcypromine) for 30 to 45 min, and (4) incubation of sections in the reaction mixture containing 10$^{-2}$M p-phenyl-isopropylhydrazine or 10$^{-4}$M tranylcypromine). In the first and second tests, the MAO reaction was almost completely inhibited in the sections. In the third and fourth tests, the MAO reaction was largely prevented. Accordingly, the tryptamine-tetrazolium method is applicable to the brain tissues of the dogfish for the demonstration of MAO activity.

In order to identify the exact localization of MAO activity in the hypothalamus, sections (10 $\mu$) stained with paraldehyde fuchsin (AF) (according to Oksche et al., 1959) or toluidine blue were compared with those showing MAO activity.

The nomenclature of Kappers et al. (1936) and of Mellinger (1963) and Meurling (1967) has been used to designate the hypothalamic nuclei and the hypophysial region.

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**Fig. 1.** Diagram showing the distribution of MAO-positive fibers in the hypothalamo-hypophysial region of the dogfish, *Triakis scylla*. The tractus strio-thalamicus et hypothalamicus (TSH) contains MAO-positive fibers, which terminate both in the nucleus medius hypothalamicus (NMH) and the neurointermediate lobe (NIL) passing through the fiber layer of the median eminence (ME). The commissure pre-infundibulaire (CPI) includes MAO-positive fibers, branches of which run into the nucleus tuberis (NT). The median eminence receives MAO-positive fibers from both the NMH and NT, especially around blood capillaries of the portal vessels (PV) in the posterior region (heavily dotted region). MAO-positive fibers from the NMH terminate in the NIL. The region with oblique lines represents the pars distalis which exhibits moderate to strong MAO activity. IS, infundibular stem; OC, optic chiasma; PPD, proximal pars distalis; RPD, rostral pars distalis; SV, saccus vasculosus; III, third ventricle.
Results

Median eminence and Infundibular stem

A diagram showing the anatomy of the hypothalamo-hypophysial region of Triakis is given in Figure 1. With AF-staining, it has been revealed that the median eminence and infundibular stem of Triakis were divisible into three layers; the ependymal layer, internal layer (hypendymal and fiber layers) and external layer, like those of the tetrapod. Some of the nerve cells of the nucleus tuberis (NT) were distributed in the anterior region of the median eminence. The internal layer contained fibers beaded with AF-positive material and running to the neurointermediate lobe. In the external layer AF-positive material was localized around the blood capillaries of the primary plexus mainly in the anterior region of median eminence. These structures are very similar to those of other species of elasmobranchs (Mellinger, 1963; Meurling, 1967).

The ependymal cells of the median eminence and infundibular stem showed slight MAO activity in their cytoplasm. The hypendymal cells gave two types of staining in their cytoplasm; one was slight and the other weak to moderate. Fibers with formazan deposits running toward the third ventricle between the hypendymal cells were sometimes observed (Fig. 2). Some fibers were in close contact with the hypendymal cells (Fig. 3). Both fiber and external layers generally

Fig. 2. Monoamine oxidase in the ependymal and hypendymal cells of the median eminence. Ependymal cells (EP) show slight MAO activity. Some of the hypendymal cells (HP) show moderate activity. The cells belonging to the NT give strong enzymatic activity (arrow 1). Note the abundant deposition of formazan in the fibers (arrow 2). III, third ventricle. × 450.

Fig. 3. Higher magnification of the infundibular stem. Note strong MAO activity around the blood capillaries (BC). Arrows show MAO-positive fibers running toward the neurointermediate lobe. EP, ependymal cells; HP, hypendymal cells; PD, pars distalis; SV, saccus vasculosus. × 450.
showed moderate MAO activity (Fig. 4). However, in the fiber layer, there were a considerable number of strongly MAO-positive fibers (Figs. 3 and 4). These fibers originate mostly in the nucleus medius hypothalamicus (NMH) and the nucleus tuberis (NT) as mentioned below. Nerve fibers around the blood capillaries of the primary plexus showed strong MAO activity (Figs. 3, 7 and 8).

**Innervation of MAO-positive fibers in the median eminence**

The *tractus strio-thalamicus et hypothalamicus* (TSH, Fig. 1) contained a large number of fibers beaded with heavy formazan deposits. Some of these fibers entered the NMH (Fig. 5) and were in contact with the cells of this nucleus (Fig. 6). A small number of fibers were branched and entered the NT. Other fibers of TSH proceeded toward the neuro-intermediate lobe through the fiber layer of the median eminence.

The *commissure pre-infundibulaire* (CPI) included MAO-positive fibers. Some of them extended toward the NT through a rather ventral part of the NMH and the lateral region of the median eminence (Figs. 1 and 4). Some of these MAO-positive fibers from CPI were in contact with the NMH-cells.

MAO-positive fibers arising in the NMH proceeded just above the AF-positive fibers in the fiber layer of the anterior region of the median eminence. In the posterior region of the median eminence, some of those fibers proceeded to the neurointermediate lobe together with AF-positive fibers, whereas others proceeded below the AF-positive fibers and terminated mainly on the central region of the median eminence, where the capillaries of the primary plexus are distributed (Figs. 1 and 7).

The NT sent MAO-positive fibers mainly to the latero-posterior region of the median eminence. Most of these fibers first ran ventro-caudally toward the fiber layer of the posterior region of the median eminence. Accordingly, some of the fibers crossed the AF-positive tract, whereas others extended along the
Fig. 6. Monoamine oxidase in the cells of the nucleus medius hypothalamicus. a: cells showing weak MAO activity in their cytoplasm (arrow), b: cells showing strong MAO activity in their perikarya (arrows). × 672.

In the infundibular stem, strong MAO activity was also observed around the blood capillaries (Fig. 3). However, the origin of these MAO-positive fibers was not ascertained.

**NMH and NT cells**

Two types of cells were distinguishable both in the NMH and NT on the basis of differences in MAO activity. The cells of one type showed slight or weak MAO activity in their cytoplasm and those of the other type showed moderate to strong enzymatic activity (Figs. 5 and 6). The cells of the latter type were stained throughout the perikarya, axons and dendrites as far as they could be traced. However, in these axons and dendrites the MAO activity was not so strong as in their endings.
MAO-positive fibers from the TSH and CPI were in contact with the NMH and NT cells, respectively, whether the cells were MAO-positive or -negative.

**Neurointermediate lobe**

The neurointermediate lobe of *Triakis* stained with AF showed a structure similar to those of the other species of elasmobranchs that have been studied (Meurling, 1962 and 1963; Knowles, 1965).

The neurohypophysial tissue contained a number of fibers beaded with heavy formazan deposits; probably the same fibers as those observed in the fiber layer of the median eminence. These beaded fibers were often observed between the intermediate cells, and sometimes terminated in close contact with them. They frequently formed neuropils. Some of the beaded fibers appeared to end on capillary walls. The intermedia cells around the sinusoid-like capillaries, which were cylindrical in shape and ocherous in color with the counterstains of AF-method, showed a rather moderate reaction for MAO in their cytoplasm. The cells within the cellular cord of each lobule of the neurointermediate lobe, which were oval and bright orange-red, showed very slight MAO activity (Fig. 9).

**Pars distalis**

Although it is not so clear, regional differences in MAO activity were observed in the pars distalis. The folliculate cells of the dorsal half of the rostral and proximal pars distalis just above the hypophysial lumen showed moderate to strong MAO activity in their cytoplasm (Fig. 4). Strong enzymatic activity in the cells of this region was observed at the periphery of the cells, in the region of the cell membrane (Fig. 8). However, it was difficult to discern whether this strong activity was due to the cell membranes of the secretory cells or due to monoaminergic terminals around them. In the proximal pars distalis, those cells situated below the lumen appeared to show
weak MAO activity throughout their cytoplasm. The folliculate cells which were located in rather ventral parts of the rostral and proximal pars distalis and weakly stained with fastgreen, showed slight MAO activity.

Discussion

It has been proposed by the present author that in the hypothalamus of the mouse and the quail those cells which exhibit moderate to strong MAO activity in their perikarya are monoaminergic neurons (Urano, 1968). The reason is that the cells showing high MAO activity in the nucleus arcuatus of the mouse and nucleus tuberis of the Japanese quail seem to be identical to those showing monoamine fluorescence observed in some species of mammals (Fuxe and Hökfelt, 1969) and the Japanese quail (Sharp and Follett, 1968). The present study shows that there are two types of cells in the NMH and NT of Triakis: cells of one type are slight to weak and those of the other type are moderate to strong in MAO activity. The cells of the latter type are probably monoaminergic neurons. This supposition is supported by the electron microscopic studies showing the presence of possible monoaminergic granules in the perikarya of the neurons of the NT of elasmobranchs (Mellinger, 1963). The distribution of MAO-positive fibers in the Triakis hypothalamus is shown in Figure 1.

There has been no investigation dealing with monoaminergic fibers in the hypothalamus of elasmobranchs, except for the suggestion that the tubero-hypophysial system in Scylliorhinus caniculus may contain monoaminergic fibers, because of the presence of electron dense granules in that system (Mellinger, 1963). The present study reveals a MAO-positive tubero-infundibular tract, which must be monoaminergic, between the NT and the median eminence. This tract is comparable to the tubero-hypophysial tract of tetrapods and the MAO positive nucleus lateralis tuberis (NLT)-neurohypophysial tract observed in the teleosts, Oryzias latipes and Anguilla japonica (Urano, 1971). It may not be correct to visualize the MAO-positive fibers between the NMH and the median eminence as belonging to the tubero-hypophysial system. The NT is mainly innervated by MAO-positive fibers from CPI, but the NMH seems to be innervated mostly by MAO-positive fibers of TSH. Therefore, it is definite that these two nuclei are different neuronal groups. Accordingly, it is difficult to name the NMH, annexed with the NT, the nucleus lateralis tuberis, a situation unlike that of the holoccephalian fish (Sathyanesan, 1965; Jasinski and Gorbman, 1966). It is probable that the MAO-positive NMH-infundibular fibers correspond to monoaminergic fibers of the "tractus hypothalamo-hypophysaire" arising from both anterior and medio-lateral regions of the hypothalamus as has been suggested by Barry (1968).

It was observed that in the NMH and NT, MAO-positive fibers terminate on the perikarya of both MAO-positive and -negative neurons. The innervation of fibers of the TSH in the hypothalamus has been reported by Kappers et al. (1936). The present study shows that these fibers of the TSH are monoaminergic and that they innervate the NMH. It has not been ascertained whether the MAO-positive fibers arising from the CPI and terminating in the NT correspond to the MAO-positive innervation in the NLT observed in the teleosts, Oryzias latipes and Anguilla japonica (Urano, 1971), or to those fluorescent fibers from the preoptic area to the NT of the Japanese quail (Sharp and Follett, 1968).

Electron microscopic studies by other investigators showed synapses between monoaminergic terminals and several types of neurons in the NLT of Oryzias latipes (Oota, 1963), nucleus arcuatus of the rat (Kobayashi et al., 1967) and the anterior hypothalamic nuclei (Iraldi et al., 1963). Considering the results obtained by the present studies and by
those investigators, it is strongly suggested that MAO-positive fibers innervating both the NMH and NT participate in the regulation of neuronal function in these nuclei.

The general distribution of MAO activity in the median eminence of *Triakis* is very similar to the distribution of catecholamines and MAO activity in avian and mammalian median eminence (Matsui and Kobayashi, 1965; Follett et al., 1966; Urano, 1968; Sharp and Follett, 1968; Fuxe and Hökfelt, 1969). The median eminence of *Triakis* receives monoaminergic fibers from the NMH and NT. The terminals of these fibers are more concentrated in the posterior region than in the anterior region of the median eminence. Sharp and Follett (1968) showed that a catecholamine-containing tubero-infundibular tract terminates principally in the posterior median eminence in the Japanese quail. MAO activity in the external layer of the median eminence in the Japanese quail (Urano, 1968) and the white-crowned sparrow (Follett et al., 1966) was also stronger in the posterior region than in the anterior region. These findings suggest that there is some monoaminergic mechanism in the median eminence, which is more developed in the posterior region than in the anterior region. Although biological significance of this phenomenon is not clear at the present time, it may be possible that these monoaminergic fibers are involved in the regulation of adenohypophysial function at least at the level of the median eminence.

In some elasmobranchs, the presence of non-neurosecretory nerve fibers entering the neurointermediate lobe has been reported by Meurling (1962 and 1963). Most of them are probably monoaminergic nerves arising in both the NMH and the TSH, since the present study shows that fibers beaded with formazan deposits are often observed between the intermedia cells and around the blood capillaries. This distribution of MAO-positive fibers in the neurointermediate lobe of *Triakis* agrees with that in the pars intermedia of the mouse (Urano, 1968) and the toad, *Bufo bufo* (Urano, unpublished data). It has been shown that fluorescent monoaminergic fibers are present in the frog, *Rana temporaria* (Enemar and Falck, 1965) and the pig and the rat (Björklund, 1968). Possible monoaminergic synapses on the cells of the pars intermedia were observed in the cat (Bargmann et al., 1967) and in the frog, *Rana pipiens* (Nakai and Gorbman, 1969). Knowles (1965) showed electron microscopically that the terminals of monoaminergic fibers are in direct contact with the intermediate cells in the elasmobranch, *Scylliorhinus stellaris*. It has been emphasized that monoaminergic fibers have an inhibitory action on the intermediate cells in the pars intermedia of the toad (Iturriza, 1969). Inhibitory effects of noraminergic fibers would also be expected in the pars intermedia of elasmobranchs.

In the pars distalis and neurointermediate lobe of *Triakis*, a considerable number of cells exhibited MAO activity. It is possible that these cells contain monoamines or monoamine-like substances. Von Euler (1961) determined high concentrations of noradrenaline and adrenaline in the pituitary gland of the dogfish, *Squalus acanthias*. Relatively high concentrations of serotonin were found in each of the three regions, pars distalis, pars intermedia and pars nervosa of rat pituitary glands (Piezzi and Wurtman, 1970). Moreover, on the basis of microspectrofluorimetric and chemical analyses, Björklund and Falck (1969) have suggested that tryptamine is present in the pars distalis of the cat pituitary.

As for the physiological role of MAO in the nerve endings, especially in the median eminence, two possibilities are considered. If monoamines act as transmitter substances at nerve terminals, MAO may protect against the excessive build-up of biogenic monoamines by deamination, in a similar manner to that of acetylcholinesterase in destroying excessive acetylcholine liberated from presynaptic nerve endings and preventing its accumulation on the surfaces of postsynaptic membranes. An alternative possibility is that enzymatically
deaminated MAO products, such as indoleacetalddehyde, 5-hydroxyindoleacetalddehyde, are biologically active, and perform some role in the median eminence. It has been established by several workers that the role of MAO in organisms is not confined only to the inactivation of biogenic amines (see Gorkin, 1966). For instance, Barondes (1962) showed that a number of amines and aldehydes have the ability to stimulate glucose-1-C14 to C14O2 by beef anterior pituitary slices and that MAO inhibitors block the effects of neuroamines but not the effects of aldehydes. He suggested that some apparent effects of amines may be mediated by their deaminated aldehydes. Recently, Sabelli et al. (1969) demonstrated that the indoleacetaldehyde and 5-hydroxyindoleacetaldehyde were still biologically active, and that they were as potent and rapid in action in the central nervous system as the corresponding amines, tryptamine and 5-hydroxytryptamine. The experiments reported in the present study show that high MAO activity is present in the neurohaemal region of Triakis. Consequently, there is the possibility that both monoamines and aldehydes are involved in the function of the median eminence in this elasmobranch.

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References


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