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RE-INVESTIGATION OF THE CONTRIBUTION OF THE SUPERIOR CERVICAL GANGLION TO INNERVATION OF THE RAT THYMUS

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In this study the contribution of the superior cervical ganglion (SCG) to the thymic sympathetic nerve supply has been re-investigated. For this purpose, two weeks after the removal of both SCG, the thymic concentrations of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) were measured neurochemically, while the distribution and density of monoaminergic nerve fibers were assessed using the method of fluorescence histochemistry. The activity of acetylcholinesterase (AChE), the occurrence of which is well documented in the sympathetic nerves, was also determined neurochemically and histochemically. The removal of SCG did not significantly affect either the thymic concentrations of NA or intrathymic distribution and density of the autofluorescent fibers. The concentration of 5-HT, as well as the density of autofluorescent cells (afc), also did not differ significantly between the experimental and control rats. The removal of SCG influenced neither the density and distribution of AChE positive fibers nor the intrathymic AChE activity. Thus the results indicate that the neural influence from the SCG on the thymus is either nonexistent or minimal.

Key words: superior cervical ganglionectomy; noradrenaline content; rat thymus

INTRODUCTION

It is well-established that the thymus of rats is innervated by fibers from the autonomic nervous system (Felten et al., 1985; Bulloch, 1985; Fatani et al., 1986). From anatomical tracing studies and cholinesterase histochemistry it seems that the thymus is innervated via branches of the vagus and phrenic nerves (Bulloch and Moore, 1981; Bulloch and Pomerantz, 1984; Bulloch, 1985). However, these results may not be conclusive as technical problems related to the spread of anatomical tracers from their sites of injection have been identified (Yu, 1980; Fox and Powley, 1985). Moreover, the occurrence of cholinesterase activity in the sympathetic nerve is also well documented (Bel-

linger et al., 1985; Burnstock, 1978). On the other hand, it has been reported that the thymus receives a substantial input from the sympathetic nervous system (Williams and Felten, 1981; Bulloch and Pomerantz, 1984) as well. After an injection of wheatgerm agglutinin-horseradish peroxidase (WGA-HRP) into the thymus, labelled cells were observed in the superior cervical ganglion (SCG) and cervicothoracic ganglia as far caudally as T3 (Nance et al., 1987). However, the source of the sympathetic input to the thymus gland might be even more restricted since the spread of the anatomical tracer to other structures cannot be completely ruled out.

Having all that in mind we attempted to evaluate the contribution of sympathetic nerve fibers deriving from the SCG to the thymic innervation. For this purpose both the SCG were extirpated and the intrathymic presence of monoaminergic and cholinergic nerve profiles were investigated using histofluorescence and neurochemical methods.

MATERIALS AND METHODS

Thymuses In the present experiment inbred AO strain rats of both sexes were used. They were housed in standard cages and maintained on a 12:00 lights on, 12:00 lights off schedule at 21 ± 1^0C. Food and water were supplied ad libitum. The superior cervical ganglia (SCG) were removed carefully (experimental group) or the skin above the ganglia was dissected (sham operated rats) and two weeks later the rats were sacrificed, their thymuses removed and stored at -20^0 C until the analyses were carried out. The operations were performed under nembutal anaesthesia (40 mg/kg).

Intact and sham operated rats of the same age as the experimental rats served as controls.

Fluorescence and enzyme histochemistry Serial sections of 10 μm thickness were cut through the thymus for 0.5 mm with approximately 20 sections separating each group of serial slides. Consecutive thymic sections were alternatively treated by fluorescence, enzyme histochemistry and classical hematoxylin-eosin staining methods. This last staining method was used to provide histological orientation and identification of the thymic compartments. Each group consisted of at least 7 thymuses.

Fluorescence histochemistry The procedure for histofluorescence was a modification of the sucrose phosphate glyoxylic acid (SPG) method (Qayyum and Fatani, 1985). The slides were immediately dipped in a solution containing 1% glyoxylic acid, 0.2 M sucrose, and 0.236 M potassium phosphate monobasic (pH 7.4). We standardized the time between cryostat sectioning and dipping in glyoxylic acid, and used two hair dryers with standardized drying time and temperature to assure comparable fluorescence. The sections were then covered with immersion oil and heated at 95^0 C for 2.5 min, drained, and immediately coverslipped. To prevent diffusion and photodecomposition of the fluorescence the sections were analyzed and photographed on the same day. An Olympus BH 2 fluorescence photomicroscope equipped with exciter filter BG 12 and barrier filter Y 495 was used to examine and photograph the sections.

Enzyme histochemistry For the demonstration of cholinergic nerve profiles, the thymic sections were fixed alternatively in 10% formalin containing 1% calcium chloride (1 min) (Patrick et al., 1980) or in 2% glyoxylic acid buffered to pH 0.2 (15 min) (Quayyum and Fatani, 1985), and then all sections were processed according to the direct colouring thiocholine method (Karnowsky and Roots, 1964). Tetraisopropylpyrophosphoramide (iso-OMPA, Sigma) was used as the inhibitor of nonspecific esterase activity. Control slides were incubated in buffer without either the substrate or the nonspecific esterase inhibitor. The thymic sections were examind and photographed under an Olympus BH2 Photomicroscope.

Determination of NA and 5-HT concentrations The monoamine levels were determined according to the fluorometric method of Laverty and Taylor (1968). Briefly, thymic samples were homogenized in acidifid butanol, and then NA and 5-HT were extracted into 0.1 N HCl. The hydroxyindol technique was used to form a fluorophore, and the intensity of fluorescence was measured on an Aminco-Bowman spectrophotofluorimeter. L-noradrenaline hydrochloride and serotonin creatinine sulphate (Koch-Light, England) were used as standards.

Determination of cholinesterase activities The AChE and butyrylcholinesterase (BuChE) activities were measured by the colorimetric method of Ellman et al. (196l). This method was designed for determination of tissue cholinesterase activity, and its main principle is that thiocholine formed during hydrolysis of the substrate rapidly reacts with DTNB (5,5'-dithiobis-2-nitrobenzoic acid) and releases a colored 5-thio-2-nitrobenzoate anion having an absorption maximum at 416 nm. The supernatant obtained after spinning the thymus homogenate was mixed with DTNB-phosphate buffered solution, and the reaction was started by adding 20 ml of acetylthiocholine iodide (1mmol/l) or butyrylthiocholine iodide (2.6 mmol/l). The increase in absorbency of the reaction mixture was read every minute (A/min) for 3-5 min at 416 nm and the mean value calculated. The ChE activity is expressed in international units (IU=nanomoles of substrate hydrolyzed per minute per gram of tissue). Since the molar absorptivity of the yellow anion is known $\varepsilon = 1.36 \times 10^4$ $mol^{-1}cm^{-1}$) the rates in absolute units can be calculated as follows:

$$IU = \frac{A}{min} \times \frac{10^3}{\varepsilon} \times \frac{\text{total volume}}{\text{sample volume}}$$

Thymuses from at least 7 animals from each group were used. The results are expressed as mean ± SEM. To assess the statistical significance of differences between means, ANOVA was used followed by Tukey's test.

RESULTS

Fluorescence histochemistry. The results clearly showed that the bilateral removal of SCG did not markedly affect either distribution or density of the thymic fluorescent nerve fibers (Figure 1). Thus autofluorescent nerve fibers were found in the capsule, interlobulary septa and among parenchymal elements of the cortex (Figure 1). Only sparse fluorescent nerve profiles were found in

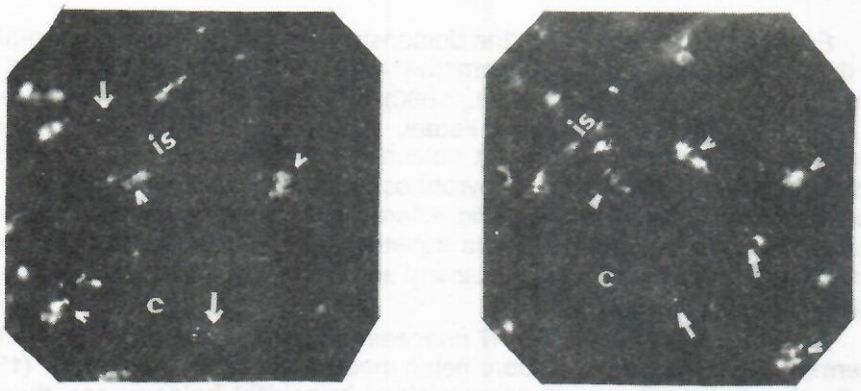

Figure 1. Photomicrographs of the autofluorescent nerve fibers (arrows) and cells (arrow heads) in the thymus of intact (left) and bilaterally ganglionectomized rats (right). X40
is = intralobulary septae; c = cortex

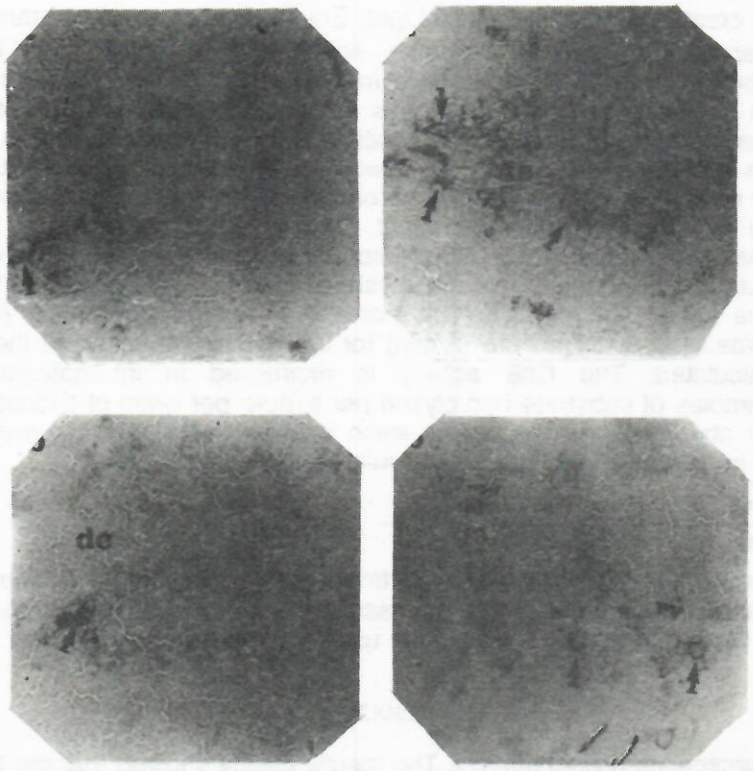

Figure 2. Photomicrographs of the AChE positive nerve fibers (arrow heads) and cells (arrows) in the a) thymic subcapsulary cortex of the intact (left) and bilaterally ganglionectomized rats (right) and b) deep cortex of intact (left) and bilaterally ganglionectomized rats (right). X 40
is = interlobulary septae; sc = subcapsulary cortex;
dc = deep cortex

the medulla. Also neither density nor intensity of fluorescence of the afc was significantly changed after the treatment (Figure 1).

Thymic NA and 5-HT concentrations. The neurochemical analysis confirmed the results obtained by the histofluorescence analysis showing that

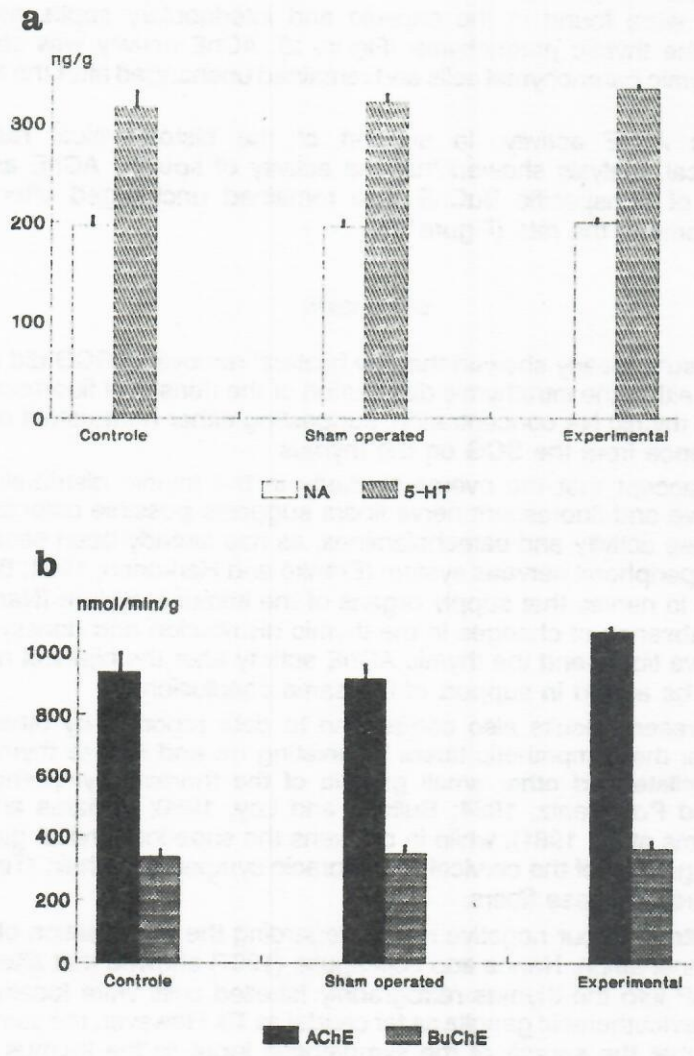

Figure 3. Concentrations of NA and 5-HT (a) and activities of AChE and BuChE in (b) the thymuses of intact, sham operated and bilaterally ganglionectomized rats. (Results are expressed as mean ± SEM, n = 7-12)

neither the thymic concentrations of NA nor 5-HT significantly differed among the experimental, sham operated or intact rats (Figure 3a).

Histochemical determination of AChE. The intrathymic distribution and density of AChE positive nerve fibers remained unchanged after the bilateral extirpation of SCG (Figure 2). Thus in all the groups of animals AChE positive nerve fibers were found in the capsule and interlobulary septa, as well as throughout the thymic parenchyma (Figure 2). AChE activity was also found within the thymic parenchymal cells and remained unchanged after the treatment (Figure 2).

Thymic AChE activity. In support of the histochemical results the neurochemical analysis showed that the activity of specific AChE as well as the activity of nonspecific BuChE also remained unchanged after bilateral ganglionectomy in the rats (Figure 3b).

DISCUSSION

The results clearly showed that the bilateral removal of SCG did not affect significantly either the intrathymic distribution or the density of fluorescent nerve fibers or the thymic NA concentration, suggesting either nonexistent or minimal neural influence from the SCG on the thymus.

If we accept that the overall similarity in the thymic distribution of the AChE positive and fluorescent nerve fibers suggests possible colocalization of cholinesterase activity and catecholamines, as has already been seen in other parts of the peripheral nervous system (Eränkö and Harkonen, 1964; Burnstock, 1978), even in nerves that supply organs of the immune system (Nance et al., 1987), the absence of changes in the thymic distribution and density of AChE positive nerve fibers and the thymic AChE activity after the bilateral removal of SCG could be added in support of the same conclusion.

The present results also correspond to data reported by other authors showing that the sympathetic fibers innervating rat and mouse thymus derive from the stellate and other small ganglia of the thoracic sympathetic chain (Bulloch and Pomerantz, 1984; Bulloch and Loy, 1980; Williams and Felten, 1981; Williams at al., 1981), while in chickens the superior cervical ganglia and other small ganglia of the cervical and thoracic sympathetic chain (Terni, 1931) are the source of these fibers.

In contrast to our negative results regarding the participation of the SCG in thymus innervation, Nance and colleagues (1987) showed that after injection of WGA-HRP into the thymus retrogradely labelled cells were localized in the SCG and cervicothoracic ganglia as far caudal as T3. However, the same authors concluded that the source of the sympathetic input to the thymus might be even more restricted than this because the spread of the anatomical tracer to other structures could not be completely ruled out (Nance and al., 1987), giving a possible explanation for this obvious discrepancy.

In conclusion, it is clear that the rat thymus receives substantial sympathetic innervation but the SCG does not directly contribute to this innervation.

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PREISPITIVANJE UČEŠĆA GORNJEG CERVIKALNOG GANGLIONA U INERVACIJI TIMUSA PACOVA

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SADRŽAJ

U ovom radu je ispitivano učešće gornjeg cervikalnog gangliona u inervaciji timusa pacova. U tu svrhu, dve nedelje nakon obostranog uklanjanja gornjeg cervikalnog gangliona, u timusu pacova je neurohemijski određivana koncentracija noradrenalina (NA) i serotonina (5-HT) i praćena gustina i distribucija monoaminergičkih vlakana metodom fluorescentne histohemije. S obzirom da je prisustvo acetilholina dokazano i u simpatičkim vlaknima, određivana je i aktivnost acetilholin esterase (AChE) i to i neurohemijski i histohemijski. Bilateralno uklanjanje gornjeg cervikalnog gangliona nije značajno uticalo ni na koncentraciju NA, a ni na distribuciju i gustinu fluorescentnih nervnih vlakana u timusu pacova. Takođe ni koncentracija 5-HT, a ni gustina autofluorescentnih ćelija, nije se značajnije menjala nakon ovog tretmana. Odstranjivanje oba gornja cervikalna gangliona nije uticalo ni na aktivnost AChE ni na distribuciju i gustinu AChE pozitivnih nervnih vlakana u timusu. Rezultati pokazuju da gornji cervikalni ganglion nije uključen u noradrenergičku inervaciju timusa.