HISTOCHEMICALLY DEMONSTRABLE
CHOLINESTERASES IN THE ADRENAL MEDULLA OF THE
HAMSTER AND THE EFFECT OF DENERVATION

By

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ABSTRACT

Acetylcholinesterase and non-specific cholinesterase gave positive histo-
chemical reactions in the nerve fibres of the adrenal medulla of the
hamster. Acetylcholinesterase activity was also observed in the fine ner-
vous network covering the whole medulla, while non-specific cholinesterase
activity was limited to fewer fibres. Division of the splanchnic nerve did
not essentially affect the non-specific cholinesterase reaction but abolished
most of the acetylcholinesterase activity. However, some fibres both in the
cortex and in the medulla retained their acetylcholinesterase activity after
denervation.

In a previous study (Eränkö 1959) it was observed that acetylcholinesterase
(AChE) has a distribution slightly different from that of non-specific cholin-
esterase (nsChE) in the adrenal medulla of the rat. Furthermore, division of
the splanchnic nerve causes an almost total loss of the AChE activity but has
little influence on the nsChE activity of the medullary fibres (Eränkö et al.
1959). In the present paper similar studies on cholinesterases of the hamster
adrenal are described.

METHODS

Experimental procedure

Adult golden hamsters were unilaterally denervated by dividing the left splanchnic
nerve under the diaphragm. The animals were killed by decapitation about 2 weeks
after the denervation. Both adrenals were removed and fixed in calcium formol for
2–4 hours. Frozen sections cut at 20 µ were rinsed in physiological saline or water and
allowed to dry on slides, mounting on the same slide sections from both the left,
denervated, and the right, intact, adrenal of each animal.
Histochemical techniques

Gomori's (1952) modification of Koelle's (1951) thiocholine method was employed for the demonstration of cholinesterase, using acetylthiocholine and butyrylthiocholine as substrates. Eserine, 62.C.47 (1:5-bis-(4-trimethylammoniumphenyl)pentan-3-one di-iodide) (Burgen 1949) and iso-OMPA (tetraisopropylpyrophosphoramide) (Aldridge 1953) were used as inhibitors (see also Pépler & Pease 1957). The sections were first incubated for 20–30 min in the inhibitor solutions and thereafter with the substrate in the presence of the same inhibitor concentration.

For the chromaffin reaction, adrenal halves were fixed in a mixture of 1 volume of 35 per cent formaldehyde and 19 volumes of 3.5 per cent potassium dichromate. The iodate reaction was carried out according to Hillarp & Hökfelt (1955). Formalin-induced fluorescence was studied as described previously (Eränkö 1955).

RESULTS

1. Normal adrenals

Nervous structures

The nerves reach the adrenal medulla through a large area of the cortex, which is penetrated by numerous straight nerve bundles separated from each others by nerve-free cortical tissue. Before entering the cortex the nerves send fibres to a narrow subcapsular network. There are also solitary fibres in the cortex, straight ones directed towards the medulla and, especially near the cortico-medullary junction, tortuous, irregularly running fibres. In the medulla there is a rich nervous network. Some of these fibres are thicker and surround the medullary cell acini and the secretery cells in them. The others are finer and form another, finemeshed network which covers the whole medulla. Along these fine fibres, small oval or round bodies are visible.

Histochemical observations

Acetylthiocholine was used as a substrate, always together with 10⁻⁶ M iso-OMPA, a combination which should selectively demonstrate AChE (see Pépler & Pease 1957; Eränkö 1959). For a presumably selective demonstration of nsChE, butyrylthiocholine and 10⁻⁵ M 62.C.47 were used. In the following description it is assumed that AChE and nsChE are thus differentiated from each other. Positive reactions were obtained with both of these substrate-inhibitor combinations, while the histochemical reaction was almost completely inhibited when both iso-OMPA and 62.C.47 were used together. However, a faint reaction was seen in many medullary fibres after incubation in a solution containing acetylthiocholine, butyrylthiocholine, 10⁻⁶ M iso-OMPA and 10⁻⁵ M 62.C.47 or, instead of these two inhibitors, 10⁻⁶ M eserine. Since this residual reaction was very weak, it was evident that the reaction observed by using either of the two above-mentioned substrate-inhibitor combinations was mainly due to true cholinesterase activity.
All the nervous structures in the cortex and both coarse and fine fibres in the medulla showed a distinct AChE activity (Fig. 1). In addition, the cytoplasm of the parenchymal cells in the medulla showed a reaction which was much less intense. Peripheral areas of the medulla were somewhat more intensely stained than those in the centre, apparently mainly because the nerve fibres were both more numerous and more reactive in the regions of the peripheral cell acini.

With butyrylthiocholine and 62.C.47, numerous nerve fibres exhibited a positive nsChE reaction (Fig. 3). However, there were fewer positive fibres, most of them coarse, both in the cortex and the medulla. On the other hand, the cytoplasm of the cortical cells in the zona glomerulosa, which remained entirely negative with acetylthiocholine, was slightly but reproducibly positive, while, conversely, the cytoplasm of the medullary cells remained negative.

The pattern formed by the medullary fibres exhibiting a positive nsChE reaction was somewhat different from that of AChE positive fibres (compare Figs. 1 and 3). In the peripheral areas of the medulla the nsChE positive fibres formed loops, presumably enclosing the parenchymal cells, but the close network formed by the fine fibres demonstrable with acetylthiocholines was not visible. The difference between the sections treated with acetylthiocholine and butyrylthiocholine was particularly clear in the central parts of the medulla. With acetylthiocholine a fine network of fibres was demonstrated there, while large areas were devoid of activity in the corresponding areas of sections incubated with butyrylthiocholine (compare lower left corners of Figs. 1 and 3).

Fig. 1.
AChE in the right intact adrenal. A strong reaction is seen in the fibres enclosing the parenchymal cells which form the peripheral medullary cell acini. The reaction is less intense in the centre of the medulla (below left), where fewer coarse fibres are present but a tight meshwork of fine positive fibres is visible. Several positive fibres can be distinguished in the cortex.

Fig. 2.
AChE in the left denervated adrenal of the same hamster. A great loss of activity is evident but some fibres are visible, both in the cortex and medulla. The photographic print has been developed somewhat longer than that in Fig. 1 to make visible the slightly positive reaction in the cytoplasm of the medullary cells.

Fig. 3./nsChE in the right intact adrenal. Positive fibres are more frequent in the region of the peripheral medullary cell acini (up right) than in the central acini (lower left corner).

Fig. 4.
sChE in the left, denervated adrenal. There is but little loss of reactive fibres and the intensity of the reaction is not much altered. Note the two cell acini above the number 4 with a positive fibre network.
Legends to the figures.

Figs. 1-4 are of the adrenal glands of the hamster. A narrow strip of cortex is seen in the upper margin of all figures. Below the corticomedullary junction is the peripheral zone of the medulla, containing in the hamster the noradrenaline-storing medullary cell acini. The central part of the medulla, composed of adrenaline-containing parenchymal cells, is visible in the lower part of each figure. Magnification in all figures is exactly the same, $\times 135$. 

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II. Effect of denervation

Denervation caused a considerable loss of the AChE reaction, most of the previously positive fibres having either disappeared or became negative. However, some clearly positive fibres were still visible both in the cortex and in the medulla (Fig. 2). These fibres had an irregular and tortuous course, and large areas in the medulla were entirely devoid of them. Ganglion cells are apparently rare in the adrenal medulla of the hamster but fibres originating from the few nerve cells observed in the denervated adrenals gave a positive AChE reaction. The cytoplasm of the medullary parenchymal cells remained positive after denervation.

The distribution of nsChE activity was, on the contrary, little affected by denervation. Positive fibres of the same type and distribution as those in the intact adrenals were also seen in the denervated glands (Fig. 4), and it was not possible to observe with certainty even a weakening in the intensity of the reaction in the fibres.

The chromaffin reaction was positive throughout the medulla both in intact and in denervated adrenals, and no distinct differences in the intensity of the reaction were observed after denervation. The same also applied to intensities of the iodate reaction and of formalin-induced fluorescence, which equally well demonstrated the presence of noradrenaline-containing cell islets in the periphery of the medulla (Eränkö 1955) before and after denervation. Comparison of the distribution of fluorescence and the cholinesterase reactions in the same section showed that the fluorescent medullary cell islets in intact adrenals are covered by a richer network of both AChE and nsChE positive fibres than the adrenaline-containing central part of the medulla.

DISCUSSION

The results obtained are principally similar with those previously reported on rat adrenals (Eränkö 1959; Eränkö et al. 1959), as far as the different distributions of AChE and nsChE and their different responses to denervation are concerned. This is in agreement with the view that AChE is the enzyme directly concerned with the secretory innervation, while nsChE apparently plays a less important role in this respect.

It is of interest that although the number of ganglion cells seems to be smaller in the adrenal medulla of the hamster, more AChE positive fibres can be seen two weeks after denervation, as compared with the rat. The morphological characteristics of these fibres suggest that they do not belong to the main secretory innervation apparatus of the medulla. Similar fibres were also seen in intact adrenals, especially near the corticomedullary junction, while in the medulla their presence was difficult to recognise owing to the dense network of secretory fibres. It might be that the denervation-resistant AChE positive
fibres take part in the control of adrenal circulation (cf. Harrison & Hoey 1960) but there is little evidence to support this view.

Other problems concerning adrenal innervation also await clarification. By improving the localising power of the histochemical techniques it should be possible to decide finally, whether the medullary fibres exhibiting a positive nsChE reaction are, as seems likely, identical with the coarser fibres which show a high AChE activity. The termination and relation of the fine AChE positive but nsChE negative fibre network to the parenchymal cells also needs clarification. We have made efforts to investigate these problems using recent histochemical methods reported suitable for the localization of cholinesterase activity even at submicroscopic level (Barrnett & Palade 1959; Lehrer & Ornstein 1959). Unfortunately, these methods proved unsuitable for the study of the adrenal medulla, which did not give a positive reaction with either method.

REFERENCES

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