Sci. Rep. Fac. Agr. OKAYAMA Univ. (52), 55–60 (1978)

# The Axoplasmic Transport of Cholinesterases in Chicken Nerves

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Recived July 1, 1978

## Summary

1. The axoplasmic transport of cholinesterases was investigated in chicken vagus and sciatic nerve trunks by the ligature method.
2. Even 48 hours after ligation, the nerve segments in which the increased activity of acetylcholinesterase (AChE) was observed were ristricted within 5mm from the ligature. The activity of AChE in these segments increased linearly until 24 hours or 48 hours after ligation.
3. Two ligatures were made on sciatic nerve trunks and the change in activity of AChE between them was investigated. And it was found that only about 13% of AChE was moved by fast flow. The velocity was 233.8mm/day in the anterograde and 97.7mm/day in the retrograde direction.
4. The activity of pseudo-cholinesterase (Ps-ChE) also accumulated with time at the proximal and distal side of the ligature.

## Introduction

In recent years, many papers were published on the axoplasmic transport of neuro-transmitters and their related enzymes. Some of them presented hypotheses concerning the physiolosical functions of axoplasmic transport. For example, the axoplasmic transport is necessary for maintainance of axon and serves to replenish the materials needed for synaptic functions. In these cases, the adequate rate of flow for the postulated functions is important. Of AChE, LUBIŃSKA *et al* and other workers reported on various aspects of the axoplasmic flow. FRIZELL *et al*[3], LUBIŃSKA *et al*[10], PARTLOW *et al*[14] and RANISH *et al*[15] also reported of the flow rate of AChE in the nerves of various animals. Since the results of these authors ranged from 5mm/day to 431mm/day, it is difficult from these values to explain the physiologic function of the axonal flow in connection with the synaptic functions. Besides, it has been reported that two kinds of AChE are present in the axon[10,12]. The discrepancy among the flow rates of AChE in the foregoing reports may be at least partly attributed to different methods and different interpretations of the results.

This investigation was performed to determine the flow rate of AChE in the vagus and sciatic nerves and to determine whether the flow rate is one kind or not.

## Materials and Methods

White Leghorn cockerels weighing 1–2kg were used. All the operations were made under sterile conditions and Nembutal anaesthesia. In vagus nerves a ligature was made at about the sixth or seventh cervical vertebra, and in sciatic nerves a ligature was made at about 3cm from *Foramen ischiadicum*. In the case of double

ligation, two ligatures were made at 2cm interval in the middle part of sciatic nerve. At an appropriate time after the operation, the birds were killed by depletion, and the nerve trunks under investigation were removed and stored at −20°C. Then the nerve trunks were cut into segments of 2mm or 5mm in length as accurately as possible under the dissecting microscope. The length of the segments was remeasured just before homogenization. The homogenization was made in an ice cooled glass homogenizer.

The activity of cholinesterases was measured manometrically with Wahrburg manometer[17]. Five mM acetylthiocholine was used as the substrate for AChE. Preincubation with 30mM iso-OMPA[17] for 30 minutes was done to inhibit Ps-ChE activity. For Ps-ChE 20mM butyrylthiocholine was used as the substrate. To eliminate simple esterase activity[7], the value obtained from the sample containing $10^{-3}$M eserine was subtracted from the value obtained from the same sample without eserine. The enzyme activity was described as $CO_2$ $\mu$l/2mm or 5mm of nerve/30 or 60 minutes. The substrates used were acetylthiocholine iodide (Sigma) and butyrylthiocholine iodide (Sigma) and the inhibitors used were eserine sulfate (Merk) and tetraisopropylpyrophosphoramide (iso-OMPA) (Koch-Light).

## Results and Discussions

1. The right-left difference and proximo-distal gradience in AChE activity of the nerves.

It is important for the accurate measurement of axoplasmic transport of AChE to know the proximo-distal gradience of the activity in nerve trunks and the difference of AChE activity between symmetrical nerves. According to LUBIŃSCA *et al*[7,9], the proximo-distal gradience of AChE activity varied with species and nerve types, and in some cases virtually no gradience was observed. Also, the symmetrical nerves are rarely identical in their anatomical and biochemical features. Consequently, if we want to use the nerve trunk of opposite side as a control, it is necessary to know forehand the difference in AChE activity between the right and the left side.

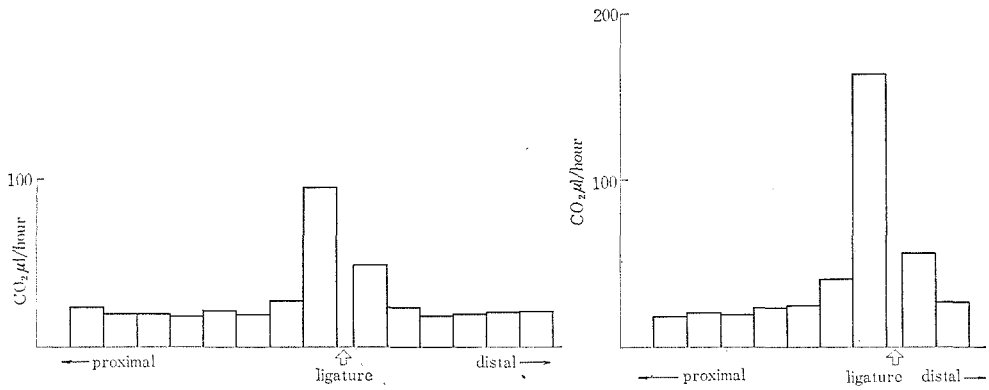
In the present experiment, no proximo-distal gradience of AChE activity was detected in the vagus nerve. In sciatic nerves the proximo-distal gradience of AChE activity was about 4%/5cm. On the other hand, no difference in AChE activity between symmetrical nerve trunks was observed in the vagus and sciatic nerve. On the basis of these findings we had determined that these nerve trunks were suitable for the experiment concerning axoplasmic transport. Therefore the symmetrical nerve trunk of the opposite side was used as a control.

2. Ranges accumulating AChE activity

At 24 hours after ligation, as shown in Fig. 1, a marked increase in AChE activity was observed in a segment just proximal to the ligature and less increase was observed in a segment just distal to the ligature. The range that accumulation occured was ristricted within 5mm from the ligature until 48 hours after ligation. These results fairly agreed with the results reported by LUBIŃSKA *et al*[8] who used peroneal nerve of dogs.

3. The change in AChE activity with time after ligation

Fig. 2 shows the changes in AChE activity with time in the just proximal and just distal 5mm segment to the ligature. After ligation AChE activity increased linearly by 24 hours in the vagus nerve, then the rate of increase lowered. In the sciatic nerve, the AChE activity in the proximal segment increased linearly by 48 hours after ligation. If the rate of flow of AChE were supposed to be one kind, the




24 hours after ligation 48 hours after ligation

**Fig. 1** The changes in AChE activity in the sciatic nerve after ligation. Each column represents the activity in 2mm segment.

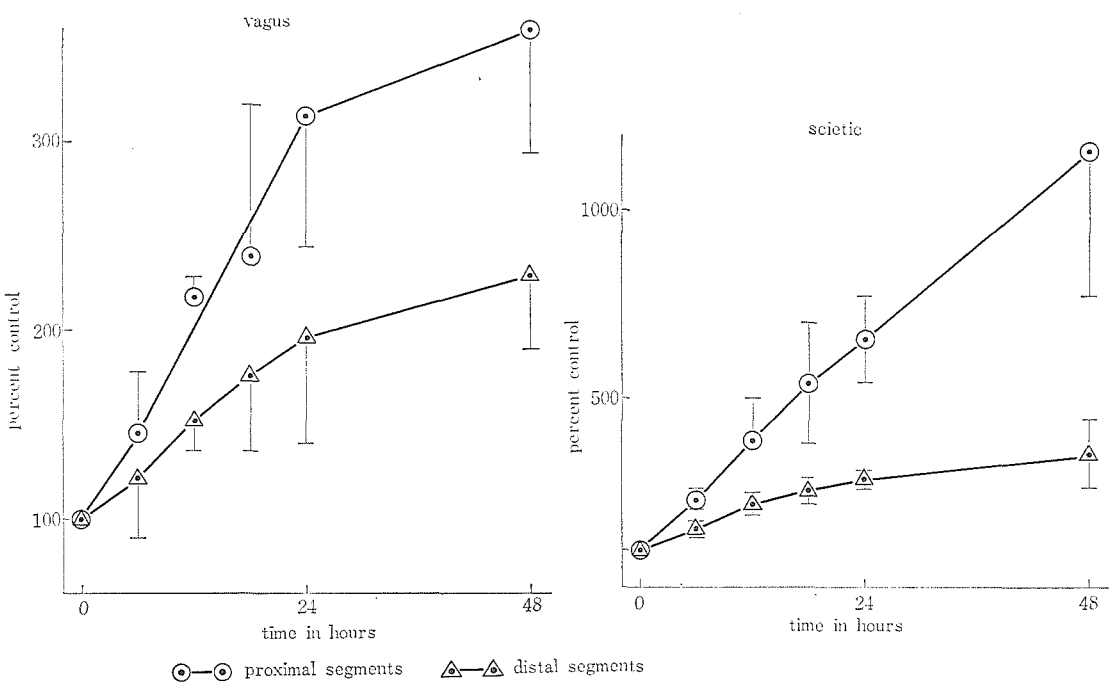



**Fig. 2** The accumulation of AChE in 5mm segments both proximal and distal to a ligature. Vertical bars represent the standard diviation.

rate of anterograde flow in the vagus nerve was 23mm/day, and that of the retrograde flow was 10.6mm/day based on the values at 12 hours after ligation. In the sciatic nerve, the anterograde flow rate was 29.1mm/day and the retrograde flow rate was 12.7mm/day. These were caluculated by the method of DAHLSTÖRM *et al*[2].

4. Two kinds of axonal AChE

The flow rates shown above were culculated, assuming that the flow of AChE in each nerve is one kind. However, we cannot determine from the results reported above that the flow is one kind. To make this problem clear, two ligatures[15] were made at 2cm interval on the sciatic nerves. AChE activities of just distal part of the upper ligature, of just proximal part of the lower ligature and of the middle part

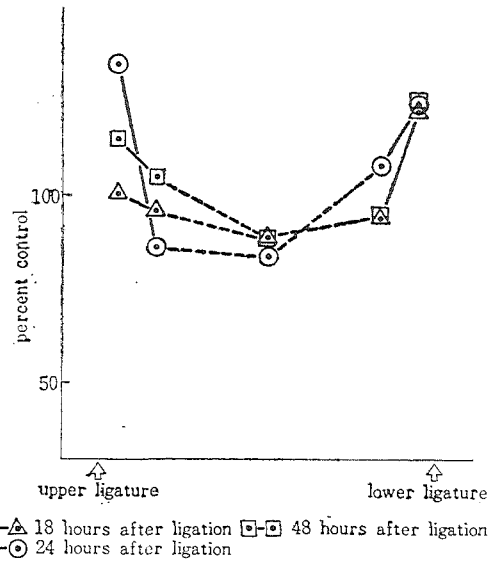



Fig. 3 The change in AChE activity with time in the nerve trunk between two ligatures. Each mark represents 2mm segment.

were determined. As shown in Fig. 3, the rise in activity was observed near the two ligatures. But the whole activity between the two ligatures was about 100% of the control value. This indicates that the whole activity did not change during the experimental period. The activities of the middle part at 18 hours, 24 hours and 48 hours after ligation were much the same and the mean of these values was 87% of the control value. Namely, in the middle part, the component to be migrated appears to deplete since 18 hours after ligation. From these results we presumed that the migrating component was about 13% of the whole activity determined ordinarily. The rest of AChE appears either to migrate quite slowly or to do not migrate at all. It was reported[13] that the material transported to the end of the axon reverses on the retrograde flow. Since the present experiment was performed in the middle part of the axon, we may disregard of this problem. The flow rate of the migrating component was 233.8mm/day in the proximo-distal direction, 97.7mm/day in the disto-proximal direction respectively. These are considered to be so-called fast flows and are similar to the results reported by LUBIŃSKA *et al*[10] who used the peroneal nerve of the dog and those reported by RANISH *et al*[15] who used the scietic nerve of the cat. On the other hand, FRIZELL *et al*[3] reported quite different results in the hypoglossal nerve (5mm/day) and in the vagus nerve (13mm/day) of the rabbit, which may be explained by the occurrence of two kinds of axonal AChE. According to LUBIŃSKA *et al*[10] and RANISH *et al*[15], about 15% of AChE activity of the nerve

trunks migrate by fast flow. Many workers admitted that the fast flow is related to microtubule. However, the result[13] contrary to this has also been reported and the relation between the fast flow and the microtubule is yet to be completely concluded. The immobile part of axonal AChE has been considered to be bound to the axonal membrane[12], but the farther studies are required on this problem.

5. The change in Ps-ChE activity with ligation

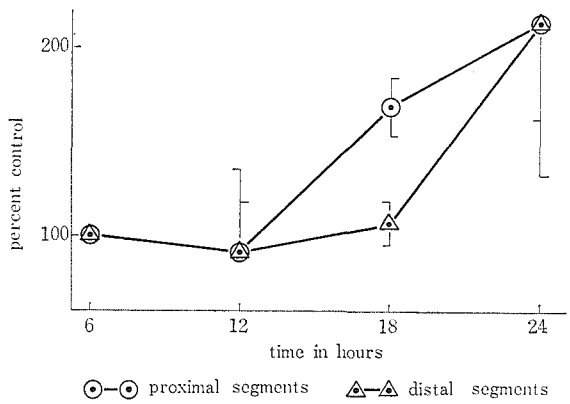



**Fig. 4** The change in Ps-ChE activity in 5mm segments both proximal and distal to a ligature. Vertical bars represent the standard diviation.

The change in Ps-ChE activity with ligation is shown in Fig. 4. At the just proximal part of a ligature, no change was observed in initial 12 hours and the accumulation of the activity has occurred since 18 hours after ligation. On the other hand, at the just distal part of a ligature, no clear change was observed until 18 hours after ligation, and then the accumulation of the activity was observed. These results seem to indicate that the activity of Ps-ChE also migrate. But the rate of the accumulation was low, we cannot eliminate the possibility that this change resulted from lesions with ligation.

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## ニワトリの神経における Cholinesterase の軸索内移動

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1. ニワトリの迷走神経と座骨神経に結紮をほどこして，Cholinesterase 活性の軸索内移動を検索した.

2. 結紮後48時間を経ても，AChE 活性の上昇の範囲は結紮点の上下 5 mm 以内にとどまった．これらの部分の AChE 活性は24時間ないし48時間後まで直線的に増加した.

3. 座骨神経に 2 cm 間隔で 2 ケ所結紮をほどこし，両結紮点間の AChE 活性の変化を経時的にしらべたところ，速い速度で移動している成分は，全活性の13%に過ぎないことがわかった．その流速は遠心性の流れで233.8mm/日，求心性の流れで97.7mm/日と計算された.

4. Ps-ChE の活性も結紮点の上下で経時的に上昇することがわかった.

# 正　誤　表 (Errata)

| 頁 (Page) | 行 (Line) | 誤 (Erratum) | 正 (Correct) |
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