鶏のCholinesteraseに関する研究 I

誌名	日本獣醫學雑誌 = The Japanese journal of veterinary science
ISSN	00215295
著者	田辺, 昭 堀, 慧 鳥海, 徹 池田, 三義 浦川, 紀元
巻/号	33巻2号
掲載ページ	p. 69-79
発行年月	1971年4月

Jap. J. vet. Sci. **33**, 69~79 (1971)

STUDIES ON THE CHOLINESTERASE OF THE CHICKEN

I. PSEUDO-CHOLINESTERASE OF THE PLASMA AND DIISOPROPYLFLUOROPHOSPHATE-RESISTANT PSEUDO-CHOLINESTERASE OF THE LIVER AND OTHER TISSUES

Akira TANABE, Satoshi HORI and Tooru TORIUMI

Department of Animal Husbandry, School of Agriculture, Okayama University, Okayama

Miyoshi IKEDA and Norimoto URAKAWA

Department of Veterinary Pharmacology, Faculty of Agriculture, University of Tokyo, Tokyo

(Received for Publication April 25, 1970)

It has been well known that the sera of various animals contain pseudo-cholinesterase. MYERS[18] mentioned that the cholinesterase of chicken serum could be classified as a pseudo-cholinesterase, mainly because the substrate activity curves of chicken serum cholinesterase against acetylcholine (ACh), propionylcholine (PrCh), butyrylcholine (BuCh) and acetyl-β-methylcholine (MeCh) were sigmoid in shape. In contrast to the results obtained by MYERS[18], BLABER and CUTHBERT[8] observed that the substrate activity curves of chicken plasma cholinesterase against ACh, BuCh and MeCh were sigmoid in shape, whereas that against PrCh was a bell-shaped curve because of the phenomenon of excess substrate inhibition. On the basis of these results, they concluded that the properties of chicken plasma cholinesterase were intermediate between those of mammalian acetylcholinesterase and those of butyrocholinesterase, and proposed that chicken plasma cholinesterase henceforth be termed the intermediate cholinesterase.

In his studies on the substrate specificity patterns of the cholinesterases in the tissues of man and animals, GOLDSTEIN[14] made it clear that the cholinesterase of the same type was present in the plasma, the liver and the gastric mucosa of the cat. MYERS[18] also found that the substrate specificity patterns of pseudo-cholinesterase were very similar in the serum, heart, and liver of the mouse, the serum and liver of the dog, the serum, pancreas, heart, and intestine of the ferret, and the serum, liver and pancreas of the chicken. On the other hand, KOELLE[15] mentioned that following incubation with 10^{-6} M diisopropylfluorophosphate (DFP) for 30 minutes, some difference was shown between the residual activity of serum cholinesterase and that of liver cholinesterase in the cat. MATSUMOTO[16] also reported that in the liver of the chicken, unlike its brain and plasma, there was a cholinesterase which hydrolyzed MeCh at a high rate and which was inhibited by 10^{-6}M DFP. In their in vivo experiment with chickens, ARDRIDGE and BARNES[2] observed that the triarylphosphates inhibited the pseudo-cholinesterase activity of the liver and intestine, but that they did not inhibit that of the plasma.

As mentioned above, there is a difference in opinions with regard to the characteristics of the cholinesterase of chicken plasma. In most of the mammals, there was no difference in characteristic between the cholinesterase of the plasma and the pseudocholinesterase of any other tissue. In the cat and chicken, opinions are still divided as to this problem. This investigation was designed to study the substrate specificity patterns of plasma cholinesterase and the difference in property between liver cholinesterase and plasma or brain cholinesterase in the chicken. The distribution of liver-type cholinesterase in other tissues and the tissue specificity and other properties of these enzymes were also studied.

MATERIALS AND METHODS

White Leghorn pullets (3~4 and 5~6 months old) and cockerels (3~4 months old) were used.

(a) *Plasma*: Heparinized blood was centrifuged at 3000 rpm for 10 minutes. The plasma was diluted ten to fifteen times with Ringer's solution. Two ml of this diluted plasma was used for each estimation.

(b) *Liver and proventriculus*: Birds were killed by bleeding. Soon after that, the tissues under investigation were collected and stored at −22.5°C. Then they were treated in the following manner. The frozen tissues were freed from clotted blood and miscellaneous tissues. They were sliced and washed three or four times with 0.9% saline. Small volumes of Ringer's solution (with 0.025M $NaHCO_3$) were added. The mixtures were homogenized by a Waring blendor. Finally the materials were homogenized by a glass homogenizer. The tissue sample in the final homogenate varied in dilution according to the activity of the tissue.

(c) *Intestinal mucosa*: Soon after bleeding, the upper half of the small intestine was harvested, cut open, and washed to be freed from food debris. The mucosa was collected by scraping the intestine. It was treated by the same procedure as employed in the liver treatment.

The activity of cholinesterase was estimated manometrically[3,21,22] at pH 7.4 and 37.5°C in Ringer's solution containing 0.025M $NaHCO_3$. The enzyme preparation was placed in the main compartment, and the substrate in the side arm. The gas phase of the manometer was filled with a gas mixture composed of 95% N_2 and 5% CO_2. After equilibration of the vessels at 37.5°C for about 10 minutes, the substrate was tipped into the main compartment. Readings were started 5 minutes later and repeated three times at 10 minutes' intervals. The enzyme activity was expressed as CO_2 μl/per gram of tissue (1 ml) per 10 minutes.

The substrates used were acetylcholine chloride (ACh) (Daiichi Seiyaku Co.), propionylcholine p-toluenesulfonate (PrCh) (NBC), butyrylcholine p-toluenesulfonate (BuCh) (NBC), butyrylcholine iodide (BuCh) (Nakarai Chemicals), and acetyl-β-methylcholine chloride (MeCh) (NBC).

The inhibitors used were diisopropylfluorophosphate (DFP) (Sumitomo Chemicals), tetra-isopropylpyrophosphoramide (iso-OMPA) (Koch-Light), and neostigmine methylsulfate (Shionogi Seiyaku Co.).

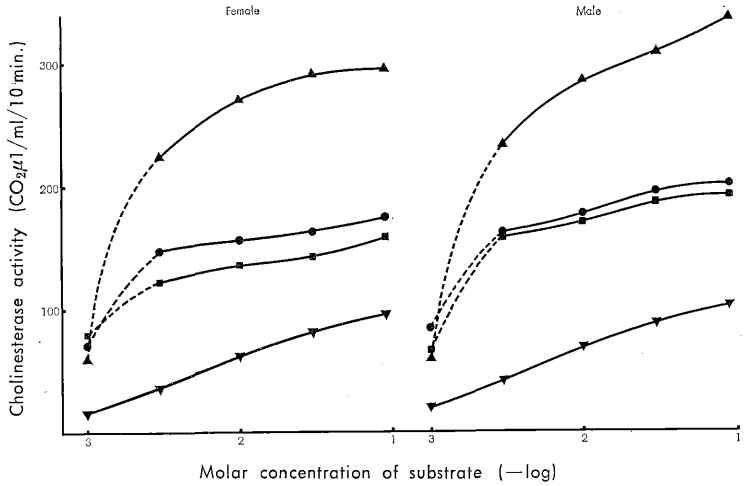
RESULTS

Fig. 1 shows the substrate activity curves of plasma cholinesterase against four substrates. The results indicate that the plasma enzyme has the highest activity over PrCh; ACh comes second; next in order are BuCh and MeCh. In the pullets, the rates of

activity over ACh, BuCh, and MeCh are 59%, 54%, and 33%, respectively, of that of activity over PrCh at a substrate concentration of 9×10^{-2}M. Similar results were obtained from the cockerels. The substrate activity curves for the four substrates were sigmoid in shape. Contrary to the results obtained by BLABER and CUTHBERT[8] PrCh showed no optimum activity in the present investigation.

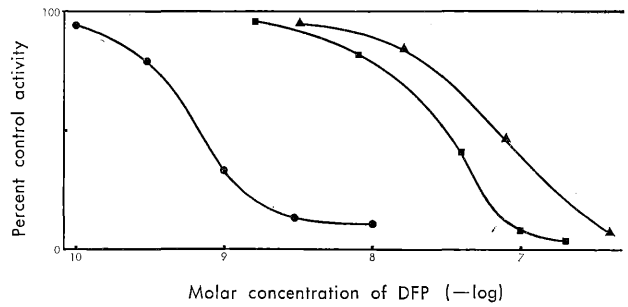
Fig. 2 shows the percent inhibition by DFP of brain, plasma, and liver cholinesterase. The activity of brain cholinesterase was inhibited by 93% with 4×10^{-7} M DFP and by 5% with 3.2×10^{-9} M DFP. The I_{50} (I_{50}=50% inhibition) concentration was about 6.8×10^{-8} M. The activity of plasma cholinesterase was inhibited by 87% with 3×10^{-9} M DFP and by 6% with 1×10^{-10} M. The I_{50} concentration was about 8×10^{-10} M. On the other hand, the activity of liver cholinesterase was inhibited by 96% with 2×10^{-7} DFP and only by 3% with 1.6×10^{-9} M. The I_{50} concentration was about 3×10^{-8} M. Thus, it was recognized that the cholinesterase of the liver was more resist-

Fig. 1. Hydrolysis of ACh, PrCh, and MeCh by Cholinesterase of Chicken



●—● ACh; ▲—▲ PrCh; ■—■ BuCh; ▼—▼ MeCh. The dotted part of the curve shows where the output of CO_2 was not linear. Chickens 3～4 months old were used in this experiment.

Fig. 2. Percent Inhibition of Brain, Plasma, and Liver Cholinesterase by DFP

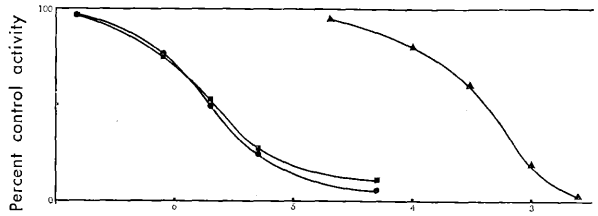


●—● Plasma; ▲—▲ Brain; ■—■ Liver. Female chickens 5～6 months old were used in this experiment. For all the three tissues 1×10^{-2}M ACh was used as substrate.

ant to DFP than that of the plasma. This agreed with the findings of MATSUMOTO[16], and the I_{50} concentration was almost equal to his result. The rate of the I_{50} concentration for brain cholinesterase to that for plasma cholinesterase was 85. The rate of the I_{50} concentration for liver cholinesterase to that for plasma cholinesterase was 38.

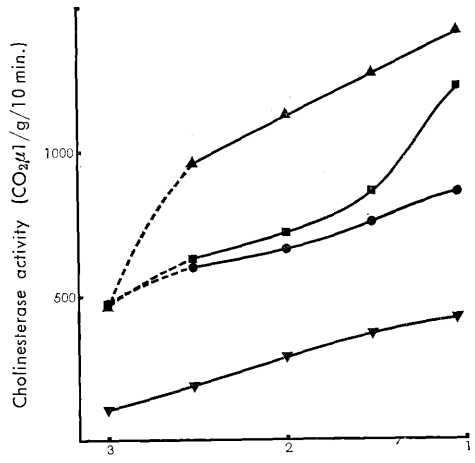
Fig. 3 shows the percent inhibition of brain, plasma, and liver cholinesterase by iso-OMPA. The rate of inhibition of brain cholinesterase was 98% by 2.5×10^{-3} M iso-OMPA and 5% by 2×10^{-5} M, and the I_{50} concentration was 4.2×10^{-4} M. Contrary to DFP, iso-OMPA inhibited liver cholinesterase to nearly the same extent as plasma cholinesterase. The activities of plasma and liver cholinesterase were inhibited by about 90% by 5×10^{-5} M iso-OMPA and by 3% by 1.5×10^{-7} M. The I_{50} concentrations of iso-OMPA for plasma and liver cholinesterase were 2.2×10^{-6} M and 1.9×10^{-6} M, respectively. The rate of the I_{50} concentration for the brain enzyme to that for the

Fig. 3. Percent Inhibition of Brain, Plasma, and Liver Cholinesterase by Iso-OMPA



●—● Plasma; ▲—▲ Brain; ■—■ Liver. Female chickens 5～6 months old were used in this experiment. For all the three tissues 1×10^{-2}M ACh was used as substrate.

Fig. 4. Hydrolysis of ACh, PrCh, BuCh, and MeCh by Cholinesterase of the Liver



●—● ACh; ▲—▲ PrCh; ■—■ BuCh; ▼—▼ MeCh. Female chickens 5～6 months old were used in this experiment. The dotted part of the curve shows where the output of CO_2 was not linear.

plasma enzyme was 191. The rate of the I_{50} concentration for the brain enzyme to that for the liver enzyme was 221. Iso-OMPA is a more distinctly selective inhibitor for plasma and liver cholinesterase than DFP.

Fig. 4 shows the substrate activity curves of liver cholinesterase against ACh, PrCh, BuCh, and MeCh. On the basis of the shapes of four curves, liver cholinesterase comes under the category of pseudo-cholinesterase. Like plasma cholinesterase, liver cholinesterase exhibits very high activity against PrCh. Contrary to plasma cholinesterase, liver cholinesterase hydrolyzes BuCh more rapidly than ACh. The rates of activity against BuCh, ACh, and MeCh are 86, 61, and 30%, respectively, of the activity against PrCh at a substrate concentration of 9×10^{-2} M. Although the activities against PrCh, ACh, and MeCh are nearly the same in both types of cholinesterase, the liver enzyme hydrolyzes BuCh more rapidly than the plasma enzyme does. This difference has a tendency to increase with the advance in concentration of the substrate.

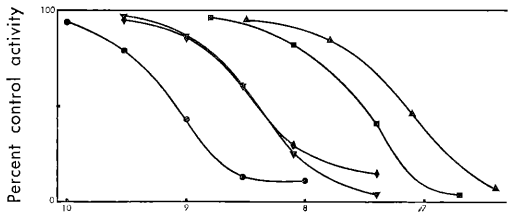
It was recognized that DFP-resistant pseudo-cholinesterase, which is similar to the cholinesterase present in the liver tissue, existed in the mucosa of the intestine and proventriculus of the chicken. It is well known that the two types of cholinesterase are inhibited by 1×10^{-6} M neostigmine, but that any other type of esterase is not inhibited by such a concentration of neostigmine[18]. It is also known that the pseudo-cholinesterases, including the chicken plasma enzyme, are inhibited by 4×10^{-9} M DFP. Table 1 shows the inhibition by DFP and neostigmine of hydrolysis of ACh

Table 1. Inhibition of Cholinesterase of the Mucosa of Intestine and Proventriculus by DFP and Neostigmine

Mucosa of intestine			Proventriculus		
Substrate / inhibitor	ACh	BuCh	Substrate / inhibitor	ACh	BuCh
—	2498±517	2855±392	—	778±154	800±203
Neostigmine	38±34	117±33	Neostigmine	29±6	
DFP		1457±337	DFP	411±59	390±47

Pullets 5–6 months old were used in this experiment.
ACh and BuCh were used at a concentration of 1×10^{-2}M. Neostigmine and DFP were used at a concentration of 1×10^{-6}M and 4×10^{-9}M, respectively. Cholinesterase activity is expressed in terms of CO_2 μl/g/10 min.

Fig. 5. Percent Inhibition of Cholinesterase of Intestinal Mucosa and Proventriculus by DFP

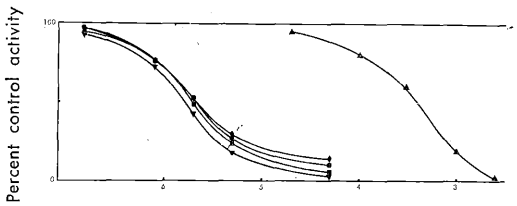


Molar concentration of DFP (—log)

●—● Plasma; ▲—▲ Brain; ■—■ Liver; ▼—▼ Intestinal mucosa; ◆—◆ Proventriculus. Female chickens 5～6 months old were used in this experiment. For all the five tissues 1×10^{-2}M ACh was used as substrate.

and BuCh in the mucosa of the intestine and the proventriculus. Results indicate that the mucosa of the intestine and the proventriculus had no other esterase hydrolyzing ACh and BuCh than cholinesterase. In the presence of 4×10^{-9} M DFP, about a half of the activity against BuCh, which is known as a specific substrate for pseudo-cholinesterase[8,18], still exists. On the basis of these facts, it is understood that DFP-resistant pseudo-cholinesterase is present also in the mucosa of the intestine and the proventriculus. Fig. 5 shows the percent inhibition of cholinesterase in the mucosa of the intestine and the proventriculus by DFP. The activity of intestinal-mucosa cholinesterase was inhibited by 96% with 4×10^{-8} M DFP, and inhibited little with 3×10^{-10} M DFP. The I_{50} concentration was 4×10^{-9} M. The rate of the I_{50} concentration for the intestinal-mucosa enzyme to that for the plasma enzyme was 5. The values in the proventricules were similar

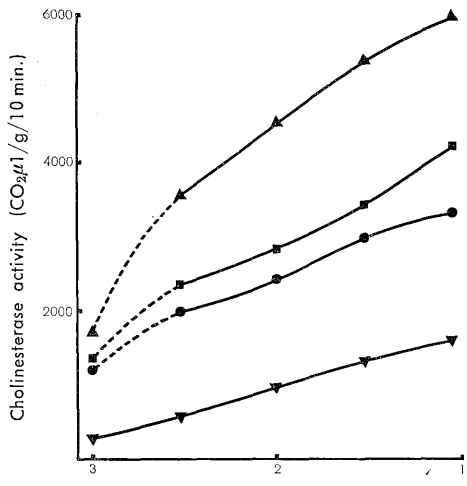
Fig. 6. Percent Inhibition of Cholinesterase of Plasma, Brain, Liver, Intestinal Mucosa, and Proventriculus by Iso-OMPA



Molar concentration of iso-OMPA (—log)

●—● Plasma; ▲—▲ Brain; ■—■ Liver; ▼—▼ Intestinal mucosa; ◆—◆ Proventriculus. Female chickens 5～6 months old were used in this experiment. For all the five tissues 1×10^{-2}M ACh was used as substrate.

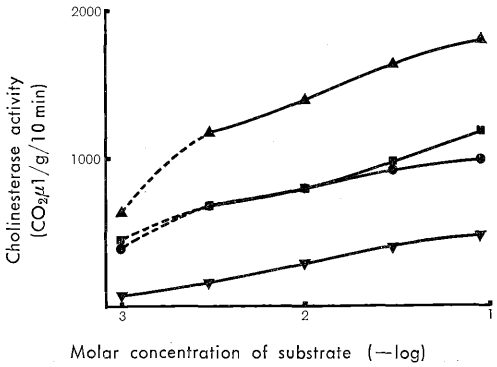
Fig. 7. Hydrolysis of ACh, PrCh, BuCh, and MeCh by Cholinesterase of the Intestinal Mucosa



Molar concentration of substrate (—log)

●—● ACh; ▲—▲ PrCh; ■—■ BuCh; ▼—▼ MeCh. Female chickens 5～6 months old were used in this experiment. The dotted part of the curve shows where the output of CO_2 was not linear.

Fig. 8. Hydrolysis of ACh, PrCh, BuCh, and MeCh by Cholinesterase of the Proventriculus



●—● ACh; ▲—▲ PrCh; ■—■ BuCh; ▼—▼ MeCh.
Female chickens 5∼6 months old were used in this experiment. The dotted part of the curve shows where the output of CO_2 was not linear.

to those in the intestinal mucosa, except that they were a little higher than the latter at a higher concentration of DFP. The I_{50} concentration for the proventriculus enzyme was equal to that for the intestinal-mucosa enzyme. Fig. 6 shows the percent inhibition of the intestinal-mucosa and proventriculus cholinesterase by iso-OPMA. Unlike DFP, iso-OMPA inhibited the intestinal-mucosa and proventriculus enzyme to almost the same extent as the plasma enzyme. The rate of the I_{50} concentration for the intestinal mucosa enzyme to that for the plasma enzyme was 0.77. The rate of the I_{50} concentration for the proventriculus enzyme to that for the plasma enzyme was 0.95. Figs. 7 and 8 shows the substrate activity curves of intestinal-mucosa and proventriculus cholinesterase against four substrates. Like liver cholinesterase, the intestinal-mucosa enzyme exhibited very high activity against PrCh. Although its activity against BuCh was not so high as in the liver, the intestinal-mucosa enzyme exhibited essentially the same activity patterns as the liver enzyme. In the proventriculus, although the activity against BuCh was still lower than in the intestinal mucosa, it was considered that the patterns of the substrate activity curves resembled those of liver cholinesterase. On the basis of the results mentioned above, it is concluded that the cholinesterase of the mucosa of the intestine and the proventriculus falls under the category of liver-type cholinesterase.

DISCUSSION

It is well known that the plasma of many animals, except the rabbit, contains pseudo-cholinesterase. In most animals, this enzyme belongs to, the so-called butyro type which hydrolyzes BuCh more rapidly than any other choline ester[18]. On the other hand, pseudo-cholinesterase of the plasma of hamster, rat, and others hydrolyzes PrCh quite rapidly. The substrate activity curves of these cholinesterases against various substrates are sigmoid in shape. In this experiment, the substrate activity curve against PrCh had no optimum, but showed a typical sigmoid shape. The ratios of hydrolysis of the four substrates practically agree with those reported by MYERS[18] It is well known that, unlike plasma pseudo-cholinesterase of various mammals, pseudo-cholinesterase of the chicken plasma hydrolyzes MeCh rather rapidly[11,16–18]. From this point,

it is certain that pseudo-cholinesterase of the chicken plasma resembles the true cholinesterase of various mammals. On the basis of the substrate activity curves, however, it is considered that cholinesterase of the chicken plasma is not an intermediate cholinesterase but a pseudo-cholinesterase. Any cause is unknown for the difference between th results of the present experiment and those reported by Blaber and CUTHBERT[8]. It is clear that there are inherited anomalous pseudo-cholinsterases, such as dibcaine-resistant pseudo-cholinesterase and sodium fluoride-resistant pseudo-cholinesterase[13]. The difference mentioned above may be due to such a inherited abnormality of pseudo-cholinesterase.

DFP has been used by many investigators as a specific inhibitor of pseudo-cholinesterase[1,9,15,19]. DAVISON[10] mentioned that the I_{50} concentration of DFP was 1.6×10^{-7} M and 2×10^{-9} M against the true cholinesterase of the chicken brain (MeCh as substrate) and pseudo-cholinesterase of the chicken plasma (BuCh as substrate), respectively. The ratio of that value to this is 80. BLABER and CUTHBERT[8] reported 24.9% inhibition of the activity of pseudo-cholinesterase of the chicken plasma with 1×10^{-9} M DFP (ACh as substrate) and 9% inhibition of the true cholinesterase of the chicken brain with 3×10^{-9} M DFP (ACh as substrate). BARON and CASIDA[7] found that the I_{50} concentration of DFP against cholinesterase of the chicken spinal cord was 1.6×10^{-7} M (ACh as substrate). According to MATSUMOTO[16], the I_{50} concentration of DFP against cholinesterase of the chicken liver was 4×10^{-8} M. The I_{50} concentration of DFP against the true cholinesterase of the chicken brain and that against pseudo-cholinesterase of the chicken plasma determined in the present experiment are half the value of the counterpart reported by DAVISON[10], although no determination was made with identical substrates. On the other hand, the I_{50} concentration against cholinesterase of the liver obtained in the present experiment is practically the same as that reported by Matsumoto[16]. He pointed out the difference between the I_{50} concentration of DFP against cholinesterase of the chicken liver and that against cholinesterase of the cat brain and plasma, but he did not compare the I_{50} concentration of DFP against the liver enzyme of chicken with that against the plasma enzyme of chicken. There is a marked difference in sensitivity to DFP between cholinesterase of the chicken liver and that of the chicken plasma. The I_{50} concentration of DFP against liver cholinesterase is near to that against brain cholinesterase.

MYERS[18] concluded that on the basis of substrate specificity patterns, the plasma, the liver, and the pancreas of the chicken contained very similar pseudo-cholinesterase. On the other hand, MATSUMOTO[16] mentioned that on the basis of its relatively low sensitivity to DFP and its relatively high affinity to MeCh, cholinesterase present in the chicken liver differed from that present in the chicken plasma. He proposed the designation of acetyl-β-methyl-cholinesterase for this enzyme. In the present experiment, the rate of hydrolysis of BuCh by liver cholinesterase was higher than that by plasma cholinesterase. These results obviously indicate that liver cholinesterase differs from plasma cholinesterase. Furthermore, on the basis of substrate activity patterns, cholinesterase of the chicken liver is apparently one of the pseudo-cholinesterases. Since it has a very high affinity to PrCh, it is to be classified as one of the propionylcholinesterases.

It was proved in this experiment that a cholinesterase similar to that present in the chicken liver existed in the mucosa of the intestine and the proventriculus. There were some differences, however, in sensitivity to DFP and in the rates of hydrolysis of the four substrates used between the two types of cholinesterase. This may be explained as follows. It has already been indicated that pseudo-cholinesterase of various animals, including the chicken, can be divided into sub-units by means of electrophoresis or

ultrafiltration[4,5,12,16,20]. Especially, REINER et al.[20] reported that the pseudo-cholinesterase of horse serum consisted of at least two sub-units which differed from each other in sensitivity to DFP. From these facts, it may be considered that cholinesterases of the chicken liver and mucosa of the intestine and proventriculus consists of several sub-units which differed from one another in sensitivity to DFP. Furthermore, all the tissues are not identical in respect to the sub-unit composition of this enzyme.

In his studies on the inhibition of cholinesterase of the central nervous system, DAVISON[10] observed that the I_{50} concentration of iso-OMPA against the true cholinesterase of the chicken brain and pseudo-cholinesterase of the chicken plasma were 4.1×10^{-5} M (MeCh as substrate) and 1×10^{-6} M (BuCh as substrate), respectively. Augustinsson[5] also reported that the I_{50} concentration of iso-OMPA against pseudo-cholinesterase of the cockerel plasma was 6.3×10^{-9} M (PrCh as substrate). The I_{50} concentration of iso-OMPA against the true cholinesterase of the chicken brain determined in the present experiment is about ten times as high as that shown by DAVISON[10]. On the other hand, the I_{50} concentration of iso-OMPA against pseudo-cholinesterase of the plasma is practically the same as that measured by DAVISON[10]. From their studies with the plasma and brain tissues of various animals, Austin and Berry[6] concluded that the treatment with 3×10^{-5} M iso-OMPA at 38°C for 30 minutes could be used to inhibit pseudo-cholinesterase without any measurable effect on the true cholinesterase. It is clear from the present studies that the treatment with iso-OMPA can be used to inhibit not only plasma cholinesterase but also DFP-resistant pseudo-cholinesterase of the liver and other tissues with no practical effect on the true cholinesterase in the chicken.

SUMMARY

1) The substrate activity curves of chicken plasma cholinesterase for acetylcholine (ACh), propionylcholine (PrCh), butyrylcholine (BuCh), and acetyl-β-methylcholine (MeCh) were sigmoid in shape. Thus it was recognized that chicken plasma cholinesterase fell under the category of pseudo-cholinesterase. Plasma cholinesterase exhibited the highest activity against PrCh, which was followed by ACh, BuCh, and MeCh in the order given with regard to this activity.

2) Cholinesterase present in the liver, intestinal mucosa, and proventriculus of the chicken was more resistant to DFP than cholinesterase in the plasma. On the basis of the substrate activity pattern, these types of cholinesterase could be classified into the same category as pseudo-cholinesterase.

3) The I_{50} concentration of iso-OMPA against DFP-resistant pseudo-cholinesterase of the liver and other tissues was practically the same as that against pseudo-cholinesterase of the plasma.

4) Unlike pseudo-cholinesterase of the plasma, DFP-resistant pseudo-cholinesterase of the liver and other tissues hydrolyzed BuCh more rapidly than Ach. These types of cholinesterase hydrolyzed PrCh most rapidly and then BuCh, Ach, and MeCh in the decreasing order listed.

REFERENCES

1) ALDRIDGE, W.N. (1953): *Biochem. J.*, **53**, 62～67.
2) ALDRIDGE, W.N. and BARNES, J.M. (1961): *Biochem. Pharmacol.*, **6**, 177～188.
3) AMMON, A. (1933): *Pflüger's Arch. ges. Physiol.*, **233**, 486～491.
4) AUGUSTINSSON, K.B. (1948): *Acta Physiol. Scand.*, **15**, suppl. 52.

5) AUGUSTINSSON, K.B. (1959): *Acta Chim. Scand.*, **13**, 1081～1096.
6) AUSTIN, L. and BERY, W.K. (1953): *Biochem. J.*, **54**, 695～700.
7) BARON, R.L. and CASIDA, J.E. (1962): *Biochem. Pharmacol.*, **11**, 1129～1136.
8) BLABER, L.C. and CUTHBERT, A.W. (1962): *Biochem. Pharmacol.*, **11**, 113～124.
9) BODANSKY, O. (1946): *Ann. New York Acad. Science*, **47**, 521～547.
10) DAVISON, A.N. (1953): *Brit. J. Pharmacol.*, **8**, 212～216.
11) EARL, C.T. and THOMPSON, R.H.S. (1952): *Brit. J. Pharmacol.*, **7**, 261～269.
12) GOEDDE, H.W., DOENICKE, A. and ALTLAND, K. (1967): Pseudocholinesterasen, pp. 7～12, Berlin, Springer Verlag.
13) GOEDDE, H.W., DOENICKE, A. and ALTLAND, K. (1967): Pseudocholinesterasen, pp. 31～54, Berlin, Springer Verlag.
14) GOLDSTEIN, A. (1951): *J. Pharmacol.*, **101**, 13～14.
15) KOELLE, G.B. (1956): *J. Pharmacol. exptl. Therap.*, **100**, 158～179.
16) MATSUMOTO, M. (1960): *Folia pharmacol. japon.*, **56**, 165～173.
17) MENDEL, B., MUNDELL, D.B. and RUDNEY, H. (1953): *Biochem. J.*, **37**, 473～476.
18) MYERS, D.K. (1953): *Biochem. J.*, **55**, 67～79.
19) ORD, M.G. and THOMPSON, R.H.S. (1950): *Biochem. J.*, **46**, 346～352.
20) REINER, E., SEUFERTH, W. and HARDEGG, W. (1965): *Nature*, **205**, 1110～1111.
21) SHUKUYA, R. (1952): Kooso Kenkyuho, part 2, p. 24, edited by AKAHORI, S., Tokyo, Asakura Shoten.
22) YOSHIKAWA, M. and UONO, K. (1961): Zoku Warburg Kenatsukei, pp. 134～140, edited, by SEKINE, T., et al., Tokyo, Nankodo Co.

鶏の Cholinesterase に関する研究

I. 血漿中の Pseudo-Cholinesterase と肝臓およびその他の組織における DFP 抵抗性 Pseudo-cholinesterase

田辺 昭・堀 慧・鳥海 徹
岡山大学農学部

池田三義・浦川紀元
東大農学部家畜薬理学教室

(昭和45年4月25日受付)

従来，鶏の Cholinesterase (ChE) に関する知見に乏しく，哺乳動物のそれとの相違点がいくつか指摘されているに過ぎない．

著者らは，鶏の ChE の特性を明らかにするため，各種の ChE 基質と二，三の ChE 阻害薬を用いて，血漿，肝臓，小腸粘膜および腺胃のChEを測定し，ChE 阻害薬の影響を調べた．

(1) 血漿中の ChE: acetylcholine (ACh), propionylcholine (PrCh), butyrylcholine (BuCh) および acetyl β methylchoine (MeCh) を基質として，血漿中の ChE 活性を測定した．哺乳動物の ChE と異なり，MeCh をかなり高率に分解することがわかった．この点では，哺乳動物の true ChE に類似する性質を有するが，上記の基質に対する酵素活性曲線は，いずれもS字状となり，いわゆる pseudo-ChE に属すると考えられる結果をえた．血漿の pseudo ChE による分解率の高い順に，上記の4基質をならべると，PrCh, ACh, BuCh, MeCh, となった．

(2) 肝臓，小腸粘膜および腺胃の ChE: 前項と同じ4基質を用いて，肝臓，小腸粘膜および腺胃の ChE 活性を測定した結果，酵素性曲線はいずれもS字状となり，pseudo-ChE と考えられた．分解率の高い順に基質をならべると，いずれの臓器においても，PrCh, BuCh, ACh, MeCh の順となり，ACh より BuCh を高率に分解することを認めた．

(3) DFP に対する抵抗性: 肝臓，小腸粘膜および腺胃の pseudo-ChE は，血漿のそれよりも DFP に対して高い抵抗性を示した．また肝臓，小腸粘膜および腺胃の間で，DFP に対する抵抗性に差があることがわかった．

(4) tetra-isopropylpyrophosphoramide (iso OMPA) に対する抵抗性: 血漿，肝臓，小腸粘膜および腺胃の pseudo-ChE は，iso-OMPA に対して，同程度の抵抗性を示した．