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ACETYLCHOLINESTERASE, CHOLINE ACETYLTRANSFERASE, AND THE POSTULATED ACETYLCHOLINE RECEPTOR OF CANINE PLATELETS*†

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Abstract—Choline acetyltransferase (acetyl-CoA: choline *O*-acetyltransferase, EC 2.3.1.6) activities of canine and human platelets were found to be surprisingly similar, in striking contrast to cholinesterase activities which differed markedly. Choline acetyltransferase activities were present predominantly in the soluble fraction of the platelet homogenate, while cholinesterase (acetylcholine acylhydrolase, EC 3.1.1.8) activities were distributed in the soluble fraction, in plasma membranes, and possibly in storage granules. In canine platelets, part of the cholinesterase activities appeared in soluble form along with adenine nucleotides and serotonin when the platelet release reaction was initiated by agents such as acetylcholine, acetyl-β-methylcholine, or thrombin; this soluble cholinesterase activity was shown to be an acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7). However, choline acetyltransferase activities were not released from platelets by these agents. Aggregation of canine platelets by thrombin resulted in a decrease of choline acetyltransferase activity in platelets by 50 per cent. Although previous results strongly suggested the presence of an acetylcholine receptor in canine platelets, demonstration of the binding of isotopically labeled acetylcholine and atropine to this postulated receptor was unsuccessful. The significance of these data and possible relationships of acetylcholinesterase, choline acetyltransferase, and acetylcholine receptor are discussed.

Cholinesterase (ChE) activities have been shown to be present in blood components such as plasma, erythrocytes, leukocytes, and platelets of different animal species [1]. Despite this, the functions of acetylcholine (ACh) and acetylcholinesterase (AChE) in platelets are still unknown. It was postulated before that AChE might play a role in the aggregation of human platelets similar to the one it was thought to play in the ACh-induced aggregation of canine platelets [2]. To date, ACh-induced platelet aggregation has been found only to be highly specific for the canine species [3]. Further study has indicated that ACh induces the release of adenine nucleotides (AN) and serotonin (5-hydroxytryptamine or 5-HT) from canine platelets [4]. This ACh-induced release reaction, as well as the aggregation reaction in canine platelets, was found to be blocked only by acetylcholine receptor (AChR) inhibitors, such as atropine, but not by AChE inhibitors, such as eserine [3,4]. Recently, we have observed the presence of choline acetyltransferase (ChAc) activity in both canine and human platelets. In the work reported here, the interrelationships of AChE, ChAc and AChR were studied, and their functional roles in ACh-induced aggregation of canine platelets investigated.

MATERIALS AND METHODS

Blood from canine, human, and various other animal species was drawn into "acid ACD" (NIH formula A) and washed platelets or gel-filtered platelets were prepared as described previously [4,5]. Washed platelets were used throughout the experiments unless otherwise indicated. ChE activities were measured by the modified radiometric method of Chuang [5] using mainly [$^{14}C_1$]acetylcholine as substrate; other substrates such as [$^{14}C_1$]acetyl-β-methylcholine (methacholine or MCh) and [$^{14}C_1$]butyrylcholine (BuCh) were used also.

ChAc activities were measured by a modified method of Fonnum [6] using Triton X-100 (5% w/v) solubilized platelets, a platelet lysate made by freeze-thaw, or a platelet homogenate obtained by shearing platelets in a glass tube with a low clearance Teflon pestle (Kontes Glass Co.). The final concentration of [$^{14}C_1$]acetylcoenzyme A (AcCoA) was reduced to 10 μM in order that the low ChAc activity in platelets could be measured. After the enzymatic reaction had proceeded to the desired point (usually 30 min), the reaction was stopped by adding cold carrier ACh (90 μg) and TCA (final concn 5%, w/v). The protein in the reaction mixture was removed by trichloroacetic acid precipitation in order to improve the separation of the organic phase (butylethylketone with sodium tetraphenyl borate 15 mg/ml) from the aqueous phase. The presence of trichloroacetic acid did not affect the final assay results. The upper organic phase (0.5 ml) was mixed with acetonitrile (2 ml) before counting in 1% phenylbiphenyloxadiapole 1,2,3-toluene mixture (10 ml) in a liquid scintillation counter (Nuclear, Chicago). The final product, [^{14}C]ACh, has not been identified. However, indirect evidence from kinetic studies shows: (a) the dependence of reaction rate upon the concentration of the substrates choline and AcCoA, (b) inhibition of the rate of product synthesis by cold ACh, and (c) reduction of the rate of

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[^{14}C]ACh synthesis in the absence of the AChE inhibitor, eserine. These findings suggest that the final product is most likely acetylcholine. Other products, such as acetylcarnitine, cannot be ruled out completely, although carnitine and acetyl-CoA: carnitine *O*-acetyltransferase have not been demonstrated in platelets.

Techniques for detection of the release reaction of the platelets by analysis of AN and [^{14}C]5-HT in the release supernatant have been described previously [4]. The release supernatant was dialyzed thoroughly against several changes of buffer (see below) before it was assayed for ChE activity.

Studies of the binding of the radioisotopically labeled ligand, [^{3}H]ACh (46 mCi/m-mole), [^{3}H]atropine (263 mCi/m-mole) or [^{14}C]*d*-tubocurarine (112 mCi/m-mole) to the postulated ACh receptor were performed by equilibrium dialysis and differential centrifugation techniques [7].

Both washed platelets and platelet plasma membrane preparations obtained by the glycerol lysis method of Barber and Jamieson [8] were suspended in Tris (30 mM, pH 7.4) buffered saline (154 mM NaCl). Radioactivity was measured by use of a liquid scintillation spectrometer (Nuclear Chicago, Mark I). Protein content was estimated by the method of Lowry *et al.* [9].

[^{14}C]ACh, [^{14}C]MCh, [^{14}C]BuCh, [^{3}H]ACh and [^{14}C]AcCoA were obtained from New England Nuclear, while [^{3}H]atropine, [^{14}C]*d*-tubocurarine and [^{14}C]5-HT were purchased from Amersham/Searle.

RESULTS AND DISCUSSION

ChE and ChAc activities of platelets of various animal species. Table 1 shows the descending order of ChE activities of platelets from various animal species. It can be seen that feline platelets possess the highest ChE activity, whereas bovine platelets have the lowest. However, as reported previously [3], among all the species tested only canine platelets consistently have shown ready aggregation in response to added ACh. Feline platelets showed inconsistent aggregation responses, while platelets from other species such as horse, goat, pig, human and cow consistently were found not to be aggregated by ACh. ChAc activities of canine and human platelets were investigated and found to be similar, although their AChE activities differed by more than 100-fold (Table 1). These results show that, while platelet AChE activity is grossly different in various animal species, ChAc activity is similar in the platelets of the two species in which it was studied here.

The ratios of the rates of ACh hydrolysis and ACh synthesis by canine and human platelets were calculated to be 1800 and 160, respectively, from the data in Table 1. These values are comparable to those for conductive tissues of various species [10]. It must be pointed out that the ChE and ChAc activities presented in Table 1 do not represent the maximal rates, because both activities were measured at reduced substrate concentrations. In addition, it will be shown later that ChE activities obtained from intact platelets represent only a part of the activity of a cell homogenate. Nevertheless, these data indicate the capability of platelets to hydrolyze ACh as well as to synthesize it.

It has been shown previously [5, 11] that the intact human platelet ChE is an AChE. It can be seen in Fig. 1 that the ChE of intact canine platelets prepared by gel filtration is an AChE also. The apparent K_m of canine platelet AChE is 143 μM which is essentially identical to that of human platelet AChE (150 μM). In addition, Fig. 1 indicates that canine erythrocyte ChE is an AChE also with an apparent K_m for ACh of 164 μM. The typical ACh substrate inhibition of canine platelets and erythrocytes is demonstrated in Fig. 1 also. On the other hand, the plasma ChE is shown to be a butyrylcholinesterase with an apparent K_m of 250 μM for ACh and 83 μM for BuCh. Additional data on the cholinesterase activities of canine platelets will be presented and discussed in Table 5.

Subcellular distribution of AChE and ChAc in canine platelets. It is known that, in other cells [1], AChE is a membrane-bound enzyme, while ChAc is found in the cytoplasm. It has been shown that AChE of erythrocytes and platelets is associated with the cell ghosts [1]; however, it has not been demonstrated before whether ChAc activities also are present in these cells. The ChE and ChAc activities of subcellular fractions of canine platelets prepared by several different techniques are shown in Table 2. It can be seen that the ChAc of canine platelets is indeed a cytoplasmic enzyme, since no activity could be detected with intact cells, and the predominant activity (70 per cent or more) was retained in the soluble fraction after platelets were lysed and centrifuged at 100,000 *g* for 1 hr. On the contrary, ChE activities were found to be widely distributed in the soluble fraction, the plasma membrane fraction, and even the storage granule fractions. It appears that the AChE activities obtained from intact cells and from plasma membrane fractions are exoenzymes and are comparable (33 vs 21 per cent, Table 2). However, the 3-fold

Table 1. Platelet ChE and ChAc activities of various animal species*

Species	ChE activity	ChAc activity
Feline	613.0 (3)	ND
Canine†	71.8 (6)	4.0 (5)
Equine	71.0 (5)	ND
Caprine	4.8 (2)	ND
Porcine	3.6 (4)	ND
Human‡	0.7 (15)	4.3 (2)
Bovine	0.4 (5)	ND

* ChE and ChAc activities were measured by radiometric methods as described in the text. Washed intact platelets were used for assay of ChE activities, and Triton X-100 (5%) solubilized platelets were used for determination of ChAc activity. ChE activities are expressed as 10^{-4} μmoles ACh hydrolyzed/min/10^8 platelets at 37° and pH 7.4. ChAc activity is expressed as 10^{-6} μmoles ACh synthesized/min/10^8 platelets at 37° and pH 7.4. Values are the average from the number of separate experiments shown in the parentheses. ND indicates that a particular entity was not determined.

† The rate of ACh hydrolysis was computed to be at least 1800 times faster than the rate of ACh synthesis by 10^8 canine platelets at 37° and pH 7.4.

‡ The same ratio for human platelets was found to be 162.

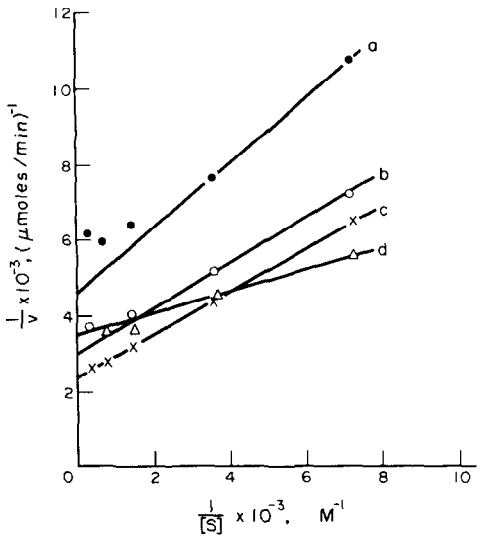

Fig. 1. Effect of ACh and BuCh concentration on the ChE activities of canine platelets, erythrocytes and plasma (Lineweaver–Burk plot). ChE activities were measured at various ACh (a, b, c) and BuCh (d) concentrations by the radiometric method described in the text. Gel filtered platelets (a, 5×10^7/ml), washed intact erythrocytes (b, 2.8×10^7/ml) and ACD plasma (c, 2.5 μl/ml; d, 5.0 μl/ml) were used for each set of assays. The apparent K_m values for (a), (b), (c) and (d) are 150, 170, 240 and 83 μM respectively. Data are representative of two separate experiments. All data in this figure were obtained simultaneously with materials from one donor.

increase in ChE activities that occurred when platelets were lysed by homogenization, by freeze-thaw, or by use of the nonionic detergent Triton X-100, coupled with the demonstration of a significant distribution of ChE activities in these other subcellular fractions (Table 2), clearly indicates the probable presence of these activities somewhere within, rather than on, the platelet. The actual location of these ChE activities within the cell and the mechanism whereby AChE activity appears in the soluble fractions cannot be determined from the present study.

Releasable and soluble ChE of canine and human platelets. ChE activities were detected in the release supernatants along with AN and serotonin during an earlier study [4]. Table 3 shows the quantitative and simultaneous release of both AN with [^{14}C]5-HT and of AN with ChE activity in studies of canine platelets by the release-inducing agents ACh, MCh and thrombin. The parallel release of these three components suggests that they may come from the same intracellular compartment. On the other hand, it can be seen in Table 4 that both ACh and MCh failed to release AN or ChE activity from human platelets. These findings agreed well with previous findings that ACh and MCh failed to induce the aggregation of human platelets [3]. The release of AN and ChE activity, as well as the occurrence of aggregation, took place with human platelets only when thrombin was used as the inducing agent. It can be seen also from Tables 3 and 4 that the thrombin-induced release of AN was comparable in canine (50 per cent) and human platelets (49 per cent), but the release of ChE activity in canine platelets (31 per cent) was much higher than in human platelets (13 per cent).

A substrate specificity study of the releasable ChE activities of canine platelets, shown in Table 5, indicated that they are AChEs, because ACh is hydrolyzed at a much higher rate than MCh (10 times) or BuCh (100 times). The specific activities (units/mg of protein) of the releasable ChE indicated a 5-fold increase over that of the platelet lysate and a 16-fold increase over that of intact platelets. ChAc activity was not detected in the release supernatants in tests with ACh, MCh or thrombin used as inducing agents. These data add further evidence to the concept that ChE activities are present within the platelet, but how

Table 2. Distribution of ChE and ChAc activities of canine platelet subcellular fractions*

Expt. No.	Fractions	ChE activity (%)	ChAc activity (%)
1	Intact cells	100	0
	Homogenization by freeze-thaw	323	87
	With Triton X-100 (5%)	306	100
2	Cell homogenization by pestle and tube grinding	100	100
	Supernatant (100,000 *g*, 1 hr)	30	78
	Sediment (100,000 *g*, 1 hr)	70	20
3	Cell homogenate by a glycerol lysis technique	100	100
	Plasma membrane	21	2
	Soluble (193,000 *g*, 3 hr)	20	70
	Sediment (193,000 *g*, 3 hr)	47	23
	Intact platelets	33	0

* ChE and ChAc activities were measured as described in Table 1. Values are expressed as per cent of cell homogenate or as indicated. Data are the averages of two to five separate determinations. Both ChE and ChAc activities may not be quantitated from the same preparation. Methods for cell homogenization were: (a) by freeze-thaw three times in a dry ice–acetone bath, (b) by Teflon pestle and glass tube homogenization for 5 min, and (c) by a glycerol lysis technique described in the text. Plasma membrane, and soluble and sediment fractions in Expt. 3 were obtained from a 27% sucrose density gradient centrifugation in a Beckman SW36 rotor at 193,000 *g* for 3 hr.

Table 3. Simultaneous release of AN, [^{14}C]5-HT and ChE activities of washed canine platelets by ACh, MCh or thrombin*

Release agents	AN released (%)	[^{14}C]5-HT released (%)	AN / 5-HT	ChE activity released (%)	AN / ChE
Simultaneous release of AN and [^{14}C]5-HT					
ACh (1 mM)	22.0	19.5	1.12		
MCh (1 mM)	24.0	23.5	1.02		
Thrombin (5 units/ml)	59.8	42.7	1.40		
Simultaneous release of AN and ChE					
ACh (1 mM)	29.7			22.7	1.31
MCh (1 mM)	27.5			21.2	1.29
Thrombin (5 units/ml)	56.8			30.7	1.85

* Release reaction of canine platelets is described in the text. ChE activities in the release supernatants were measured after dialysis. Data are presented as net per cent of AN released from the platelets, net per cent of [^{14}C]5-HT released from prelabeled platelets, and net per cent of cholinesterase activity released from platelets (cells homogenized by freeze-thaw were used to obtain the 100 per cent value). Data are representative of two to four separate experiments.

these enzyme activities are made available during the platelet release reaction is unknown.

Effect of aggregation on canine platelet ChAc activity. The effect of thrombin-induced platelet aggregation on the ChAc activity of canine platelets is shown in Table 6. It can be seen that ChAc activities were reduced when platelets aggregated in response to thrombin regardless of whether platelets were suspended in buffer or in plasma. However, this reduction in ChAc activities is greater in plasma (50 per cent) than in buffer (10 per cent). Apparently, the presence of plasma protein produces two effects: (a) it reduces the ChAc activity in the assay system (e.g. 24 per cent reduction in control 1) probably due to competition for the substrate molecule AcCoA (10 μM) by ChAc, plasma proteins, and plasma enzymes such as AcCoA hydrolase, and (b) there is an enhancement of platelet aggregation by certain plasma proteins that might influence ChAc activities.

Thrombin, on the other hand, produced several effects in addition to the aggregation of canine platelets. It can be seen in Table 6 that, in the control assays (control 1 and 2), thrombin inhibited ChAc activity (32 per cent) only when plasma proteins were present with platelets in the assay mixture. This suggests that the effect of thrombin on ChAc in the presence of plasma protein could be due to thrombin-induced alteration of one or more plasma proteins. Thrombin is a protease and is known to degrade several different plasma proteins such as fibrinogen. Nevertheless, the reason for the reduction of ChAc activities after platelet aggregation induced by thrombin is not known. Whether this reduction of ChAc activities is due to inhibition by metabolites such as cAMP, cGMP, or prostaglandins generated and released during platelet aggregation, or whether there is an as yet unrecognized feedback control mechanism for the regulation of ChAc activity within the platelets after their aggregation are both possibilities that cannot be evaluated at the present time. Furthermore, the role of plasma proteins or other plasma constituents in the additional reduction of ChAc activities (from 10 to 50 per cent) is not clear and requires further investigation.

ACh and the release reaction and aggregation of canine platelets. Eserine, a cholinesterase inhibitor, when present at a concentration of 50 μM was shown earlier to inhibit all of the platelet ChE activity present. On the other hand, eserine did not seem to affect the ACh-induced release reaction [4] or aggregation [3] of canine platelets. However, what effect the presence of eserine might have on the fate of added ACh during aggregation and release was previously unknown. Whether the hydrolysis of ACh is essential for the ACh-induced functional changes of release and aggregation was therefore examined. Table 7 shows that the release from canine platelets of AN by ACh (1.0 mM) or by [^{14}C]ACh (1.3 mM) in the presence of eserine sulfate was essentially the same (23 per cent). The recovery of ACh as [^{14}C]ACh following the release reaction was quantitative (98.2 per cent), while the recovery of the breakdown product, [^{14}C]acetate, was minimal (1.8 per cent). However, in the absence of eserine, degradation of [^{14}C]ACh is obvious, since the recovery of [^{14}C]ACh was only about 3 per cent, while the product, [^{14}C]acetate, was

Table 4. Simultaneous release of AN and ChE activities from washed human platelets by thrombin*

Release agents	AN released (%)	ChE activity released (%)	AN / ChE
ACh (1 mM)	0.2	0.0	
MCh (1 mM)	0.3	0.0	
Thrombin (5 units/ml)	48.7	12.5	3.9

* All conditions were the same as in Table 3 except that human platelets were used. Data are averages of two separate experiments.

Table 5. Substrate specificity and specific activity of ChE activities of intact canine platelets, a platelet lysate, and the release supernatant*

Fractions	ChE as per cent of lysate	Substrate specificity ACh/BuCh	Substrate specificity ACh/MCh	Sp. act. ($\times 10^{-2}$)
Platelet lysate	100	94	11	7.7
Intact platelets	33.8	115	15	2.5
ACh release supernatant	22.3	98	11	37.9
MCh release supernatant	19.6	106	10	41.1
Thrombin release supernatant	34.4	99	13	44.1

* Release supernatants were prepared as described in Table 3. Platelet lysate was prepared by freeze-thaw. The assay using [^{14}C]BuCh or [^{14}C]MCh as analogous for [^{14}C]ACh was as described in the text. Protein was estimated by the method of Lowry *et al.* [9]. Data are representative of four separate experiments.

predominant (96 per cent). The release of AN by [^{14}C]ACh (1.3 mM) in the absence of eserine, on the other hand, was found to be only slightly less (20 per cent) than when eserine was present (23 per cent). It can be seen also that atropine (10 μM), which is a specific inhibitor of AChR, blocked the release of AN completely. Further, choline, which is one of the breakdown products of ACh, could not replace ACh in induction of the release reaction.

The fate of ACh in ACh-induced canine platelet aggregation was studied in a manner similar to that used for investigation of the release reaction. [^{14}C]ACh was added to platelet-rich plasma in both the presence and absence of eserine to initiate platelet aggregation which was measured by aggregometry. After aggregation was completed (3–5 min), [^{14}C]ACh and [^{14}C]acetate were analyzed. Essentially the same data were obtained as are shown in Table 7.

These results clearly demonstrate that: (a) intact ACh is essential for the initiation of release and aggregation in canine platelets, (b) the hydrolysis of ACh is not directly related to the release reaction, (c) intact platelet AChE may not be involved directly in ACh-induced release and aggregation, and (d) the ACh-induced release reaction is most likely mediated by an AChR.

Postulated AChR of platelets. Data from Table 7 and data from previous studies [3,4] have suggested strongly the presence of an AChR on the canine platelet plasma membrane. However, efforts to demonstrate such a receptor by studying the binding of [^{3}H]ACh (substrate) or [^{3}H]atropine or [^{14}C]*d*-tubocurarine (AChR inhibitors) to intact platelets and to platelet plasma membrane preparations by an equilibrium dialysis technique or by the differential centrifugation method have been unsuccessful. This failure may be due to the following reasons: (a) the concentration of AChR as indicated by the low AChE activity in platelets in comparison to other tissue cells is too low to be detected, (b) the specific activities of the radioisotopically labeled compound employed were too low to show significant counts even though binding did occur, and (c) the binding of ligand to AChR may be highly reversible.

Binding of [^{125}I]α-Bungarotoxin, an irreversible blocker for nicotinic receptors, was not tried since α-Bungarotoxin was found not to inhibit platelet

Table 6. Effect of cell aggregation on the ChAc activities of canine platelets*

Experimental conditions	ChAc activity (10^{-6} μmoles/min) Platelets in buffer + $CaCl_2$ (5 mM)	ChAc activity (10^{-6} μmoles/min) Platelets in citrated plasma
Control 1: no aggregation, no thrombin added	3.95	3.00
Control 2: no aggregation, thrombin added after Triton	4.12	2.05
Test: aggregation with thrombin added before Triton	3.67	1.05

* Canine platelets were suspended in either citrated plasma or a Tris–saline buffer. In tests, thrombin (5 units/ml) was added to initiate platelet aggregation and the mixture was incubated further for 5 min at 37°. Triton X-100 (1%) was added to stop the reaction and ChAc activity was measured. In control 2, thrombin was added after Triton X-100. Activities were expressed as 10^{-6} μmoles ACh synthesized/min/10^{8} platelets. Data are representative of four separate experiments.

Table 7. Fate of ACh during the release reaction of canine platelets in the presence and absence of eserine*

Release agents	Net AN released (%)	[^{14}C]ACh recovered (%)	[^{14}C]acetate recovered (%)
[^{14}C]ACh (1.3 mM) + eserine (50 μM)	23.2	98.2	1.8
[^{14}C]ACh (1.3 mM)	19.8	2.7	95.8
ACh (1.0 mM) + eserine (50 μM)	23.3	†	†
ACh (1.0 mM) + eserine (50 μM) + atropine (10 μM)	1.6	†	†
Choline (1 mM) + eserine (50 μM)	1.0	†	†

* Release reactions of washed canine platelets were carried out simultaneously by addition of either ^{14}C-labeled ACh (1.3 mM) or non-labeled ACh (1.0 mM) with and without added eserine sulfate (50 μM). The release supernatants were then separated and analyzed for AN by the spectrophotometric method and for residual [^{14}C]ACh and [^{14}C]acetate by methods used to assay ChAc and ChE activities respectively. Atropine sulfate (10 μM) was used to inhibit the release reaction, and choline (1 mM) was used to replace ACh as a control. Platelet concentration was 7.7×10^9/ml in a mixture composed of 30 mM Tris (pH 7.4), 154 mM NaCl, and 5 mM $CaCl_2$. All values for AN reflect subtraction of the value of a blank with eserine. Data are representative of two separate experiments.

† No data were given since no radioactive agent was used.

aggregation or the release reaction induced by ACh [3,4]. Other specific agents, such as quinuclidinyl benzilate (QNB), have not been tested.

Final comments. The AChE of intact platelets was found not to be directly involved in the ACh-induced release and aggregation of canine platelets (Table 7). However, the simultaneous release of AChE activity with AN and serotonin (Table 6) suggests that this releasable AChE may be closely related to the function of ACh in canine platelets. How this AChE is released and where the releasable AChE activity originates are presently unknown. It was thought at first that AChE in the release supernatant might be present as a result of the solubilization of plasma membrane AChE by proteases and other lysosomal enzymes that are liberated also during the release reaction. Our evidence seems to contradict this hypothesis, because the simultaneous release of proteases and lysosomal enzymes likely would result only in the inactivation of AChE. It has been shown before that human platelet AChE is readily destroyed by proteases [11]. Direct evidence to refute the hypothesis may be obtained in the future from two different approaches: (a) release reactions should be performed in the presence of protease inhibitors to minimize the effects of released lysosomal hydrolytic enzymes, and (b) a comparative study of membrane-bound AChE and releasable AChE should be carried out to determine whether these two enzymes differ in their physical and chemical properties.

It is generally assumed that the binding of ligand to receptor on the cell plasma membrane might be the primary step in triggering various actions of the platelet. Little is known about the membrane receptors of platelets. Recently, ADP binding to isolated platelet membranes [7], thrombin binding to intact human platelets [12–14] and phytohemagglutinin binding to platelets [15] have been demonstrated. The binding to plasma membrane of other agents important to platelet function such as collagen, catecholamines and 5-HT has not been demonstrated. It seems likely that ACh-induced release and aggregation of canine platelets are mediated by an AChR, although such a receptor has not yet been demonstrated clearly. As discussed above, this is most likely due to the complexity of the platelet membrane and the technical problems involved in the study of receptors. Similar difficulties were encountered also by workers interested in the receptors of other cells as pointed out recently by Cuatrecasas [16].

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