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The effect of beta-adrenoceptor blocking drugs on 'ecto-ATPase' activity of rat blood platelets

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The mechanism of action of beta-adrenoceptor blocking drugs in affecting blood platelet aggregation is still controversial. It seems that this effect is not related to the beta-blocking activity but rather to the nonspecific alteration of platelet membrane [1, 2]. Since platelet 'ecto-ATPase' or the ability of whole washed blood platelet suspensions to dephosphorylate ATP is considered to be involved in the mechanisms of platelet aggregation [3–6], this study examined the effect of ten beta-adrenoceptor blocking drugs on the 'ecto-ATPase' activity of rat blood platelets.

Blood was collected from male Wistar albino rats (350–400 g) in ether anesthesia via polyethylene cannula from common carotid artery into plastic tubes containing citrate anticoagulant (9 ml blood + 1 ml 129 mM trisodium citrate). Platelet rich plasma (PRP) was obtained by centrifugation at 200 *g* for 20 min at room temperature. The platelets were separated by centrifugation at 900 *g* for 20 min at room temperature and washed two times with citrated saline solution (12.9 mM trisodium citrate, 137 mM sodium chloride, pH adjusted to 6.5 with HCL). The washed packed platelets were then resuspended in a Tris-buffered saline (50 mM Tris–HCl in 137 mM NaCl, pH 7.4) and platelet count adjusted to 5×10^8/ml (counted manually). Aliquots from platelet suspension were incubated with beta-adrenoceptor blocking drugs at 37° for 5 min and then mixed with an equal volume of medium containing ATP (50 mM Tris–HCl, 137 mM NaCl, 2 mM ATP, 4 mM $MgCl_2$, pH 7.4). Mixing of these solutions represented the beginning of the enzyme assay. The latter was terminated at 15 min by addition of the reaction solution (ammonium molybdate 8.09 mM, ammonium metavanadate 2.01 mM, sodium dodecylsulphate 69.3 mM, ammonia 13.2 mM, nitric acid 650 mM) according to Lin and Morales [7]. The extinction of colour complex formed by released inorganic phosphorus (P_i) and molybdovanadate was measured at 350 nm using a cell with a 1 cm path. Ecto-ATPase activity was defined as nmole P_i cleaved from added ATP per 10^9 platelets per hr.

The average ATPase activity (± S.E.M.) of a suspension of intact washed rat blood platelets was 365.1 ± 7.5 nmole P_i per 10^9 platelets per hour. The activity was linearly proportional to time and platelet concentration (Fig. 1). It was not inhibited by oubain (0.5 mM) and was about twice as great at 37° (365.1 ± 7.7) as at 22° (188.4 ± 4.6). The enzyme showed divalent cation dependence with magnesium and calcium (Fig. 2). Stimulation by magnesium was greatest at 2 mM and stimulation by calcium increased with increasing concentration up to 4 mM. At optimal magnesium concentration addition of calcium diminished the ecto-ATPase activity.

Figure 3 demonstrates the effect of alprenolol (AB Hässle, Sweden), atenolol (ICI, Macclesfield, U.K.), doberol (Boehringer, Ingelheim, F.R.G.), Ko 1124 (Boehringer), metipranol (SPOFA, Praha, Czechoslovakia), oxprenolol (Ciba-Geigy, Basel, Switzerland), pricoron (VULM, Modra, Czechoslovakia), pronethalol (ICI) and propranolol (ICI) on the ecto-ATPase activity of rat blood platelets. No appreciable changes in the enzyme activity were observed after 5 min incubation with beta-adrenoceptor blocking drugs at a concentration of 0.1 mM. Increasing the concentration of tested drugs to 1 mM led

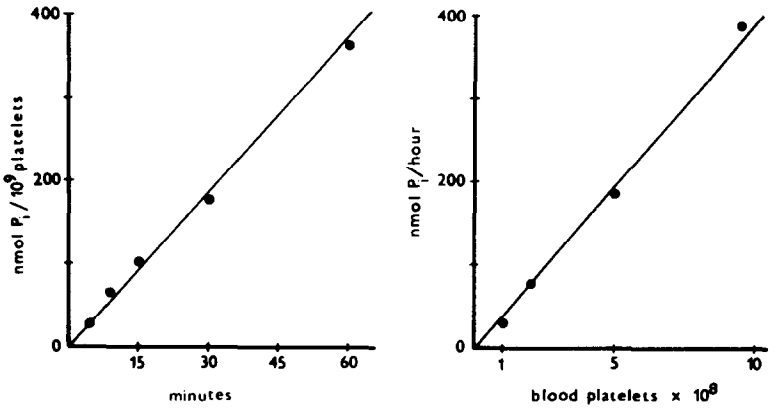

Fig. 1. Linearity of ecto-ATPase assay with time and platelet concentration. Presented results are representative results obtained in 5 experiments.

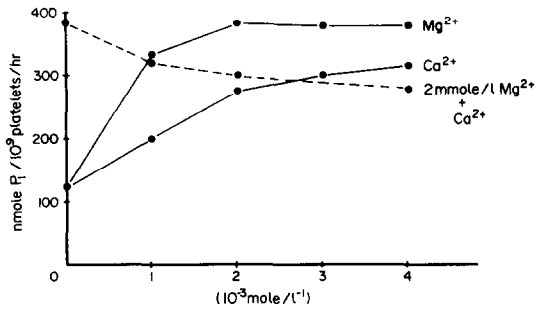

Fig. 2. Effect of variation in magnesium and calcium concentration on rat blood platelet ecto-ATPase activity. Presented results are representative results obtained in 5 experiments.

to statistically significant differences in the amount of cleaved P_i. In this concentration alprenolol, doberol, Ko 1124, metipranol, oxprenolol, practolol, pronethalol and propranolol decreased the ecto-ATPase activity of rat blood platelets as compared with control. The most effective were Ko 1124 (58.8%) and pronethalol (67.4%). On the other hand atenolol and pricoron in the same concentration increased the amount of released P_i to 420.3 ± 17.5 nmole P_i per 10^9 platelets per hr and 713.7 ± 13.8, respectively.

Beta-adrenoceptor blocking drugs owing to their physico-chemical properties interact with membranes phospholipids resulting in changes in the fluidity of biological membranes [8–10]. Physical state of membrane lipids can influence the motility and conformation of intrisic membrane proteins and thus their functions as receptors, channels, enzymes, etc. [11, 12]. Observed changes in ecto-ATPase activity after incubation with alprenolol, atenolol, doberol, Ko 1124, metipranol, oxprenolol, practolol, pronethalol and propranolol could be, therefore, explained as a results of beta-adrenoceptor blocking drugs-induced alterations in the fluidity of blood platelet plasmatic membrane. Vehement increasing in the amount of cleaved P_i after pricoron treatment was probably not due to its direct stimulatory effect but rather to the liberation of soluble intracellular ATPase activities. It was previously demonstrated that pricoron in the concentration 1 mM impairs the integrity of rat blood platelets [13].

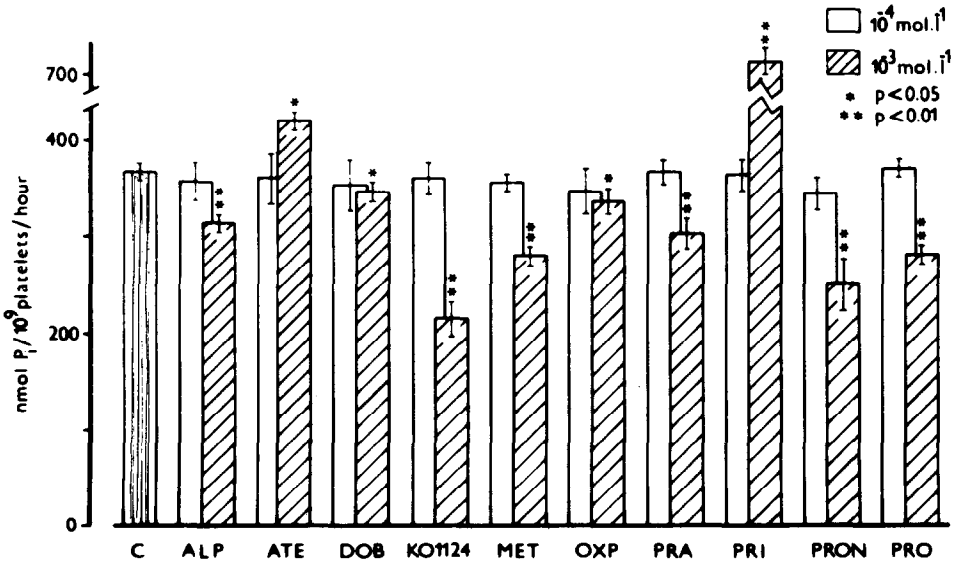

Fig. 3. Effect of 5 min incubation with alprenolol (ALP), atenolol (ATE), doberol (DOB), Ko 1124, metipranol (MET), oxprenolol (OXP), practolol (PRA), pricoron (PRI), pronethalol (PRON) and propranolol (PRO) on the ecto-ATPase activity of rat blood platelets. Data are presented as mean values from 8 independent measurements ± S.E.M.

Beta-adrenoceptor blocking drugs influence aggregation of blood platelets in concentrations 0.001 mM and more [14–16]. In our experiments concentrations required for affecting ecto-ATPase activity were 100-fold higher. These data suggest other mechanisms are responsible for the action of beta-adrenoceptor blocking drugs on the blood platelet aggregability than alterations in the platelet ecto-ATPase activity. Such mechanisms may be related to the availability of aggregation receptors, calcium availability and to the intraplatelet cyclic nucleotide levels. Further studies with platelets for elucidation of this problem are under way.

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Dichloroacetate tissue concentrations and its relationship to hypolactatemia and pyruvate dehydrogenase activation

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Dichloroacetate (DCA) activates the pyruvate dehydrogenase complex (EC 1.2.4.1) (PDH) by inhibition of PDH kinase [1, 2]. Inhibition of this regulatory enzyme allows unopposed activation of PDH by PDH phosphatase. Administration of the drug to humans [3, 4] or laboratory animals [5, 6] causes prompt lowering of blood glucose by increased utilization of gluconeogenic precursors in peripheral tissues [7]. DCA lowers blood lactate, and with chronic administration this effect persists for days following discontinuance of the drug despite the relatively short plasma half-life of DCA [3, 4, 8]. Basal PDH activity remains increased for 12–24 hr following chronic DCA administration and total PDH activity remains increased for 24–48 hr [9]. The prolonged effects of DCA are not due to its metabolites, oxylate and glyoxylate, because they do not activate PDH *in vitro* [9]. The purpose of this study was to determine tissue concentrations of DCA and how they relate to the prolonged effects of the drug.

Adult Sprague–Dawley rats, 200–250 g, were used throughout the study and had free access to water and Purina rat chow.

DCA was administered by gastric intubation, 100 mg/kg in a solution of 200 mg/ml of saline. For single dose experiments, DCA was given and three animals were killed at 1, 3, 6, 12 and 24 hr following administration. In chronic experiments, 100 mg/kg of DCA was given as a single daily dose by gastric intubation for 7 days. Three animals were then killed at 3, 6, 12, 24, 48 and 72 hr after the final dose. The animals were sacrificed under sodium pentobarbital anesthesia and exsanguinated via the abdominal aorta. Livers were excised rapidly and freeze-clamped in liquid nitrogen and stored at −70° until assayed. In some animals, muscle tissue was similarly obtained and stored.

PDH was assayed both at basal activity and following *in vitro* activation by methods previously reported [9, 10]. The percent activation was calculated as basal PDH/total PDH × 100. To determine DCA tissue concentrations, the tissue was homogenized in 10 vol. of distilled water for 30 sec in an Ultraturrax tissuemizer at full speed. It was then sonified for three 30-sec periods using a Branson sonifier. The homogenate was then centrifuged at 5000 *g* for 15 min. The concentration of DCA in the supernatant fraction was assayed using electron capture gas chromatography by the method of Sanello [11] as modified by Wells *et al.* [3]. All *in vitro* experiments were performed in duplicate.

DCA was obtained from the Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. [^{14}C-1]Pyruvate was from the New England Nuclear Corp. (Boston, MA) and all other chemicals were from the Sigma Chemical Co. (St. Louis, MO).

Statistical significance between control and treated groups was determined by Student's *t*-test.

Following a single dose of 100 mg/kg DCA, tissue concentrations of DCA increased to a maximum concentration