Effect of catecholamines on ADP aggregation of rat platelets in vivo

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Summary. The influence of catecholamines on the platelet count was studied in an in vivo experimental model obtained with cannulation of the carotid and of the femoral vein. The i.v. infusion of epinephrine and l-norepinephrine induces a low drop in the platelet count and also potentiate aggregation by ADP.

Several studies have been conducted in vitro on the effects of catecholamines on platelet function, showing that epinephrine and l-norepinephrine induce aggregation in man, but not in rat, rabbit, guinea-pig, cat, dog, horse and pig platelets[1-3]. Moreover they potentiate aggregation induced by ADP in all species considered[3-5]. These effects seem to be mediated by α-adrenergic receptors, since they are prevented by α-adrenergic receptor blocking agents[6,7]. The use of β-receptor blocking agents does not antagonize the effect of catecholamines on platelets[5], nor does it produce an unspecific inhibition at high concentrations[6,7]. As a contribution to the knowledge of the influence of catecholamines on blood platelets in vivo, in the present paper we report preliminary results obtained in the rat employing the technique already described by Kobayashi and Didisheim[8].

Methods. Adult albino Wistar rats were anaesthetized with Na nembutal and ethyl urethane. The left carotid was cannulated with polyethylene cannula for collection of arterial blood samples as previously described[9,10]. The left femoral vein was cannulated for injection of drugs and connected to an infusion pump (Harward Apparatus Co. Millis, Massachusetts, USA, Model 940). Heparin in saline (0.25 mg/ml; Fluka AG, Chemische Fabrik, Buchs SG, Switzerland) was injected to prevent formation of fibrin fibres in the cannulas; the total amount of heparin injected was about 0.40 mg. A mixture of epinephrine (10 μg/ml, ISM, Milan, Italy) and l-norepinephrine (10 μg/ml Noradrec Recordati, Milan, Italy) was infused at the speed of 0.786 ml/min. The infusion lasted about 1 min and 2 ml/kg b.wt. were injected. In some experiments, the infusion of the mixture of catecholamines lasted about 30 sec and was immediately followed by the ADP injection which lasted about 1 min (Na_3ADP, 0.427 mg/ml, 0.854 mg/kg b.wt, C. F. Boehringer & Söhne, Mannheim, BRD). Blood samples (20 μl) were collected with Unopette Disposable Pipetting System (Becton, Dickinson, France). 2 samples were collected before the infusions, to determine the basal platelet count, then other samples were collected at intervals of time and the percentual drop in the platelet count compared to the basal number was calculated. The curves shown in the figures represent the means and SE from 6 experiments for each experimental condition studied. The results were statistically analyzed with the 2 sample t-test for the limiting value of 0.01 probability.

Results. The i.v. infusion of a mixture of epinephrine and l-norepinephrine (10 + 10 μg/ml, 2 ml/kg b.wt) induces a low drop in the platelet count ($-14.79 \pm 1.53\%$, figure 1, curve b). In contrast, the infusion of ADP (0.427 mg/ml, 2 ml/kg b.wt) is followed by the well-known diminution in the platelet concentration[9-11] which reaches $-43.74 \pm 1.40\%$ at 2 min and is almost completely reversed at 30 min (figure 2, curve a). When the mixture of catecholamines is rapidly (30 sec) injected, immediately before the ADP infusion, the maximal degree of aggregation is not significantly modified ($-44.84 \pm 1.99\%$; figure 2, curve b) but the recovery appears to be slower. In fact, the percentual number of platelets still aggregated at 4 min is higher that in the control. At this time the difference

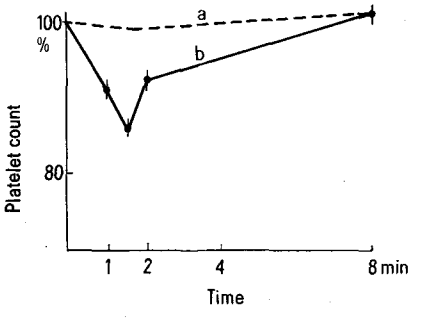

Fig. 1. Effect of the infusion of a mixture (epinephrine + l-norepinephrine 10 μg + 10 μg/ml; 2 ml/kg b.wt) on the basal platelet count. *a* Saline; *b* mixture.

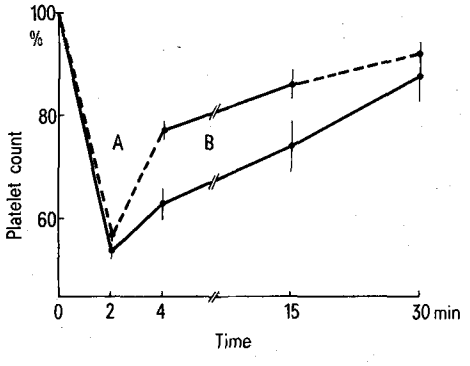

Fig. 2. Effect of infusion of solutions on the basal platelet count: *a* ADP (0.427 mg/ml, 2 ml/kg b.wt); *b* mixture of epinephrine + l-norephrine (10 μg + 10 μg/ml; 2 ml/kg b.wt) for 30 sec, immediately followed by ADP for 1 min. *A* Area over curve a; *B* area over curve b and including area A.

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between curve b and curve a is statistically significant: the t calculated was 3.659 > 3.250 for p = 0.01 probability. These results point to a potentiation provoked by catecholamines on ADP-induced platelet clumping in vivo, evidenced by a slowing down of the disaggregation. This effect of catecholamines can be further shown by the calculation of the area delimited by curve a (area A = ADP) and curve b (area B = catecholamines + ADP). In fact if we consider that the rectangular area delimited by time (30 min), in abscissa, and 100% in platelet count, in ordinate, represents the total amount of responsive platelets in the animal, it appears that the area over each curve represents the percentual of platelets which have been aggregated, and the area below each curve represents the percentual of free circulating platelets. In this way it was calculated that area B (catecholamines + ADP) is 24.34% of the total area, whereas area A (ADP) is 16.30% of the total area. Comparing the 2 areas in absolute values, it appears that B is about 50% larger than A, thus indicating a potentiation.

Discussion. Present results show that the infusion of a mixture of catecholamines in the rat, causes a low drop in the basal platelet count and also potentiates aggregation induced by ADP. Literature[1-3] indicates that aggregation by epinephrine and l-norepinephrine in vitro is present in humans but not in rats, and this discrepancy with our results points to the differences often occurring between in vitro and in vivo experiments. On the other hand, our results confirm the in vitro finding that catecholamines potentiate aggregation by ADP[4,5,7]. These findings give further evidence that there are species differences in the response of platelets to aggregating agents. Mills[3] ascribed the different behaviour of human platelets, compared to those of rats or of other animals, to the greater amount of ADP contained by human thrombocytes in the 'nucleotide storage pool'. This fact may also explain the mechanism of action underlying potentiation: authors suggest that it may involve α-receptors[5-7] and that may be mediated by endogenous ADP released by platelets in the extracellular medium[12]. Really our results show that the in vivo potentiation induced by catecholamines on ADP clumping, does not consist in increasing the maximal extent of aggregation, but in slowing the recovery. This phenomenon indicates a persistence of platelet aggregates in blood, which may be circulating, or may be filtered by some districts, for example lungs or kidneys, as previously suggested[9,13].

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Effect of pancreatic polypeptide on DNA-synthesis in the pancreas[1]

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Summary. Bovine pancreatic polypeptide increases DNA-synthesis in the rat pancreas; no effect is observed in stomach (oxyntic area), duodenum or liver. BPP neither augments or inhibits the trophic action of cholecystokinin.

Regulation of growth in the digestive tract is one of the important physiological actions of gastrointestinal hormones. Gastrin has been shown to promote the growth of acid-secreting gastric mucosa[2], while cholecystokinin (CCK) increases DNA-synthesis in pancreatic acinar tissue[3,4]. In contrast, secretin[5] and more recently motilin[6] have been shown to inhibit pentagastrin-stimulated growth in the stomach.

A trophic role for pancreatic polypeptide (PP), a new hormone found primarily in the pancreas, has not hitherto been investigated. Because PP appears to conteract CCK-mediated pancreatic enzyme secretion[7], a similar inhibitory effect on pancreatic growth might be anticipated. The purpose of this study was therefore 2fold: first to establish if PP had any trophic effects in the gastrointestinal tract and second to observe whether it could influence the trophic action of CCK.

Materials and methods. 48 male Sprague-Dawley rats (100–120 g) were fasted for 24 h in individual metabolic cages. Water was provided ad libitum. They were randomly divided into 4 groups (n = 12 for each group) and injected once i.p. as follows: Group I (control): NaCl, group II: 18 nmoles/kg cholecystokinin-octapeptide (CCK-OP), group III: 12 nmoles/kg bovine pancreatic polypeptide (BPP) and group IV, a combination of II and III. This dose of BPP has previously been shown to approximately halve CCK-OP-induced pancreatic trypsin output in the dog[7]. The volume of injection was equal in the 4 groups. After 15 h, 0.5 mCi/kg ^{3}H-thymidine (5 Ci/mmole) was injected and the animals sacrificed at 16 h. The liver, pancreas, duodenum and oxyntic area of the stomach were quickly removed, frozen in liquid nitrogen and stored at −20 °C.

Tissue samples were homogenized in 5% trichloracetic acid (1 ml/100 mg of tissue) at 4 °C. The homogenate was centrifuged and the pellet washed twice with 3 ml of 5% TCA. The DNA-containing pellet was then suspended in 3 ml of 5% TCA and heated for 10 min at 90 °C. This suspension was centrifuged and the supernatant saved. The pellet was then resuspended in 1 ml of 5% TCA and again heated and centrifuged. The supernatants were combined and incorporation of ^{3}H-thymidine into DNA determined by counting 1 ml of the supernatant in 10 ml of scintillation fluid (Instagel) in an Intertechnique scintillation counter. The DNA-content of the samples was determined using calf thymus DNA as a standard[8].

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