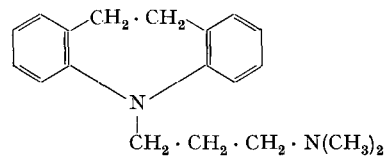
The Effect of Drugs Inhibiting the Uptake of Biogenic Amines on Adrenalin Induced Platelet Aggregation

From other authors' experiments we know that adrenalin shortens the bleeding-time and the half-time of thrombocyte survival[1-4] and that, like an injury of the vessel or an injection of cholesterol, it reduces the number of circulating thrombocytes, probably by means of the observed microthrombi formation[5]. Recently O'BRIEN, MITCHELL, and SHARP have shown that adrenalin induces platelet aggregation in vitro[6,7].

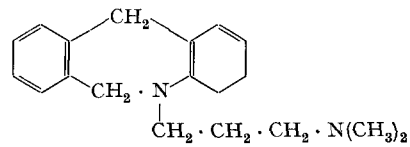
Adrenalin is presumed to induce platelet aggregation in penetrating into thrombocytes and in liberating ATP[2]. ATP is immediately decomposed into ADP, which is presumed to be the proper aggregation factor. Were this true, the substances inhibiting adrenalin uptake would necessarily block platelet aggregation as well. If they were not able to block platelet aggregation, we would have to suppose that adrenalin acts directly in the cell membrane or that it interferes with the clottable protein. For this study we chose antidepressive drugs of imipramine type, i.e. imipramine (IP), norimipramine (NIP), propazepin (P), amitriptyline (AT) and nortriptyline (NT). Their chemical structures are given in Figure 1. These drugs block the uptake of biogenic amines, including adrenalin[8]; they possess no marked adrenolytic potency, but rather enhance the peripheric nervous effect of adrenalin[8,9].

We used platelet-rich human plasma prepared according to BORN[10]. The measurement itself was performed according to O'BRIEN[6] with a slight modification: apart from the continuous drop of extinction, we found another one after 240 sec following an additional 1 min centrifugation at 25 rpm. During the procedure there appeared only tiny flakes; their dimensions excluded any influence of plasma viscosity upon their sedimentation and we obtained more consistent results. The aggregation data are given in %. The extinction drop following addition of adrenalin in a $5 \cdot 10^{-5} M$ concentration was taken as 100% aggregation.

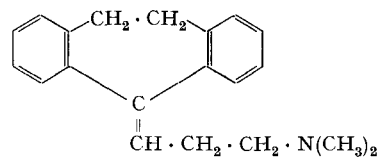
The results are summarized in Figure 2. Its upper, middle and lower parts show the effects of the drugs men-



Imipramine

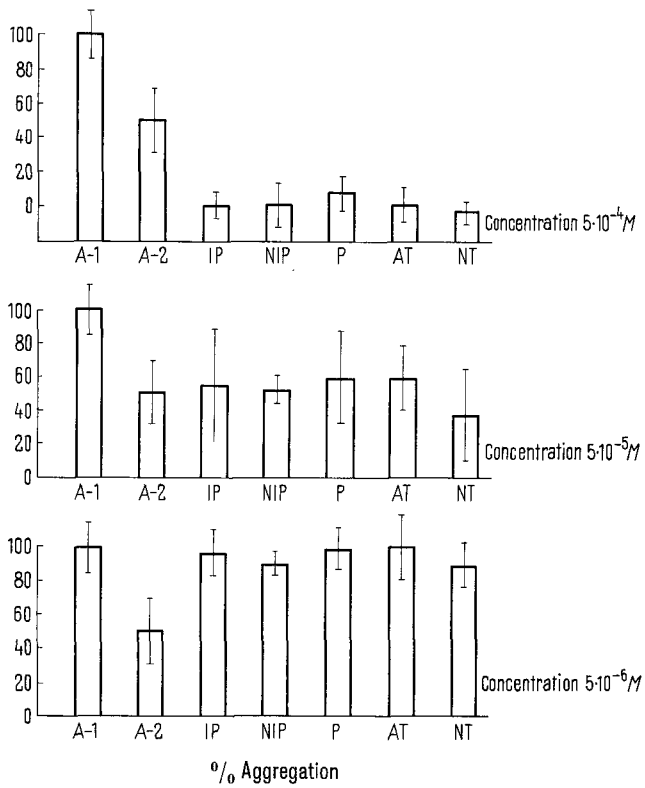


Propazepine



Amitriptyline

Fig. 1



Action of investigated compounds ($5 \cdot 10^{-4} M$ concentration) on adrenalin induced platelet aggregation (A in $5 \cdot 10^{-5} M$ concentration): Imipramine (IP) = − 0.62%, s = 7.16, n = 6; Norimipramine (NIP) = + 1.51%, s = 13.50, n = 8; Propazepin (P) = + 5.85%, s = 11.94, n = 6; Amitriptyline (AT) = − 1.39%, s = 10.83, n = 4; Nortriptyline (NT) = − 4.41%, s = 4.91, n = 10.

Action of investigated compounds ($5 \cdot 10^{-5} M$ concentration): Imipramine (IP) = + 54.36%, s = 35.56, n = 15; t (IP: A1) = 4.365456, $P < 0.001$. Norimipramine (NIP) = + 47.08%, s = 7.71, n = 8; t (NIP: A1) = 10.163437, $P < 0.001$. Propazepin (P) = + 54.71%, s = 29.14, n = 12; t (P: A1) = 5.044609, $P < 0.001$. Amitriptyline (AT) = + 58.31%, s = 29.22, n = 10; t (AT: A1) = 4.534528, $P < 0.001$. Nortriptyline (NT) = + 36.12%, s = 28.68, n = 11; t (NT: A1) = 7.182532, $P < 0.001$.

Action of investigated compounds ($5 \cdot 10^{-6} M$ concentration): Imipramine (IP) = + 97.10%, s = 14.99, n = 10; t (IP: A1) = 0.485998, $P < 0.7$. Norimipramine (NIP) = + 91.19%, s = 6.50, n = 7; t (NIP: A1) = 1.630334, $P < 0.2$. Propazepin (P) = + 99.04%, s = 12.63, n = 10; t (P: A1) = 0.174495, $P < 0.9$. Amitriptyline (AT) = + 100.98%, s = 19.40, n = 10; t (AT: A1) = 0.144246, $P < 0.9$. Nortriptyline (NT) = + 95.62%, s = 13.16, n = 10; t (NT: A1) = 0.780488, $P < 0.5$.

Fig. 2. Adrenalin (A1) $5 \cdot 10^{-5} M$ = 100%, s = 16.19, n = 14; Adrenalin (A2) $5 \cdot 10^{-6} M$ = 50,2%, s = 19.31, n = 22.

tioned in $5 \cdot 10^{-4} M$, $5 \cdot 10^{-5} M$ and $5 \cdot 10^{-6} M$ concentrations.

As we can see from the graph, these drugs inhibit the adrenalin induced platelet aggregation completely in the $5 \cdot 10^{-4} M$ concentration. In the $5 \cdot 10^{-5} M$ concentration this inhibition is still statistically significant, but there is practically no effect in the $5 \cdot 10^{-6} M$ concentration. There are no statistically significant differences between the respective drugs, yet nortriptyline and norimipramine seem to be the most potent. Their higher effectiveness is in keeping with the fact that nortriptyline and norimipramine are the most potent inhibitors of adrenalin uptake as well[9,11].

As can be seen from our results, the investigated drugs in the concentration of $5 \cdot 10^{-5} M$ reduce the action of adrenalin to $^1/_{10}$ of its real concentration (Figure 2, middle part).

The fact that the drugs used inhibit the adrenalin induced platelet aggregation proves that adrenalin acts in keeping with O'BRIEN's theory[6]: it penetrates the cells and liberates ATP from platelets, ATP converts into ADP which is the proper aggregation factor.

Zusammenfassung. Anti-Depressiva vom Imipramin-Typ, die die Aufnahme der biogenen Amine blockieren, hemmen auch gleichzeitig die durch Adrenalin bewirkte Thrombozyten-Aggregation. Dieser Befund unterstützt die Theorie des Mechanismus der Thrombozyten-Aggregation durch Adrenalin: Adrenalin dringt in die Zellen ein und setzt ATP aus den Thrombozyten frei, das sofort in ADP – den eigentlichen Aggregationsfaktor – umgewandelt wird.

K. RYŠÁNEK, C. ŠVEHLA,
H. ŠPÁNKOVÁ, and M. MLEJNKOVÁ

Medical Clinic of Postgraduate Medical Institute, and Research Institute for Experimental Therapy, Praha (Czechoslovakia), November 12, 1965.

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Balance Studies on Free and Bound Pantothenic Acid in Humans after Administration of Calcium Pantothenate or Coenzyme A

Introduction. Infusions containing coenzyme A, α-lipoic acid and diphosphopyridine nucleotide have been administered intravenously to patients with hepatic coma. During therapy the state of consciousness was improved and a decrease of blood pyruvic acid and the fraction acetoin and 2,3-butylene glycol towards normal values could often be observed[1,2]. It is assumed that these effects might be at least partly due to coenzyme A[3], which is involved in the oxidative breakdown of pyruvate (synthesis of acetyl coenzyme A) and acts as an acyl carrier in metabolism (e.g. in vivo formation of acetylcholine).

Nothing is known about the fate of coenzyme A after intravenous infusion (oxidation, breakdown, penetration into cells, excretion). In the present study, blood and urinary concentrations of free and of bound pantothenate (= coenzyme A) have been determined before, during, and after administration of coenzyme A. Similar balance studies have been made after infusion of calcium pantothenate.

Methods. (a) Laboratory investigations: In blood and urine, free pantothenic acid and coenzyme A (= bound pantothenate) were determined microbiologically with *Lactobacillus plantarum* (ATCC 17-5 8014) according to the method of SKEGGS and WRIGHT[4], coenzyme A after previous degradation to pantothenic acid with alkaline phosphatase and pigeon liver peptidase as proposed by LIPMANN[5].

(b) Infusions: Coenzyme A or calcium pantothenate was administered intravenously to convalescent patients with normal glomerular filtration rates. The infusions contained 250 μM coenzyme A (Farmochimica Cutolo-Calosi, Naples, Italy) or 250 μM D-pantothenic acid in the form of the calcium salt (F. Hoffmann-La Roche Inc., Basel, Switzerland) dissolved in isotonic glucose or saline (240 ml) and were given at a rate of 1 ml/min for 4 h. The coenzyme A content of each preparation was determined microbiologically. The coenzyme A was further characterized with the phospho-transacetylase-test and the hydroxyacyl-coenzyme-A-dehydrogenase-test[6].

(c) Collection of blood and urine samples: Before, during, and after administration of coenzyme A or calcium pantothenate, the blood level and the daily urinary excretion of coenzyme A and pantothenic acid were checked. Venous blood samples were drawn immediately before and 2, 4, $8^1/_2$ and 24 h (1 case), 2, 4 and 24 h (1 case), 24 h (1 case) or 24 and 72 h (3 cases) after the beginning of the infusion.

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