fect is more important than the first, and it ultimately leads to a fall of QC and depression of EPP during frequency testing. In turn, the change in permeability of the nerve endings for sodium causes lengthening of the relative refractory period of the nerve fibers, and during frequency stimulation this leads to omissions of AP and, correspondingly, of EPP.

We do not know whether diphenhydramine causes exhaustion of transmitter reserves in synapses of noncholinergic nature in the CNA. However, the sedative action of diphenhydramine may be partly associated with a decrease in the reliability of conduction along central nerve fibers as a result of blocking of ionic channels.

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INHIBITION OF PLATELET AGGREGATION BY ANTIOXIDANTS

K. O. Muranov, S. B. Gashev,
L. D. Smirnov, A. A. Shvedova,
V. B. Ritov, and V. E. Kagan

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One way of influencing thrombus formation in the blood vessels is by controlling platelet aggregation with the aid of chemicals. In this connection interest in new compounds with antiaggregating activity *in vitro* is increasing. In the modern view, platelet aggregation is the final stage of a complex sequence of reactions, triggered by the action of a stimulus on the plasma membrane. The molecular mechanism of the platelet aggregation process has not yet been finally established. It is considered that an important role in the aggregation process is played by cyclo-oxygenase (COG), which catalyzes the formation of prostaglandin endoperoxides. Evidence in support of this view is given by the high ability of specific inhibitors of COG, namely aspirin and indomethacin, to inhibit platelet aggregation both *in vitro* and *in vivo* [6, 7]. It has frequently been suggested that substances capable of terminating free-radical stages in reactions of oxidation of biological substrates may be inhibitors of the COG reaction. It has in fact been shown that some antioxidants have an inhibitory action of platelet aggregation [2, 3]. However, no direct proof of a connection between the antiaggregating activity of antioxidants and the COG reaction has yet been obtained.

The aim of the investigation described below was to determine whether the antiaggregating activity of antioxidants is connected with their possible effect on COG activity or whether their effect is realized by other mechanisms.

EXPERIMENTAL METHOD

Platelets were isolated from the blood of healthy donors by the method in [5]. Aggregation was recorded on an aggregometer (Chronolog Corp., USA). Aggregation was initiated with arachidonic acid (50 μM), thrombin (1.5 unit/ml), and the Ca^{++} ionophore A23187 (1.5 μg/ml). The substances for testing were added to a platelet suspension in the form of a

Institute of Chemical Physics, Academy of Sciences of the USSR. Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Faculty of Biology, M. V. Lomonosov Moscow University. Institute of Physiology, Sofia, Bulgaria.

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TABLE 1. Antiaggregating Activity of Compounds Tested

Compound	ED_{50}, M			$K_7 \cdot 10^4$, $mole^{-1}$/sec*
	arachidonic acid (50 μM)	thrombin (1.5 unit /ml)	A23187 (1.5 μg/ml)	
Indomethacin	$5 \cdot 10^{-5}$	10^{-3}	$6{,}3 \cdot 10^{-4}$	—
Ionol	$3 \cdot 10^{-5}$	$6{,}3 \cdot 10^{-6}$	$1{,}7 \cdot 10^{-5}$	—
3-HP	—	—	—	0,1
2-Benzyl-3-HP	10^{-3}	$7{,}9 \cdot 10^{-4}$	10^{-3}	0,6
2,6-Dimethyl-3-HP	—	$5{,}0 \cdot 10^{-3}$	$1{,}4 \cdot 10^{-3}$	6,5
2-Tert-butyl-3-HP	—	10^{-2}	10^{-2}	4,2
2-Ethyl-6-methyl-3-HP	—	10^{-2}	10^{-2}	8,5
3-Dimethylcarbamoylhydroxypyridine	10^{-2}	10^{-2}	10^{-2}	—
2,4,6-Trimethylpyridine	—	10^{-2}	10^{-2}	—
2-Phenyl-4,6-dimethyl-5-HPM	—	10^{-4}	10^{-3}	0,2
6-Phenyl-5-HPM	—	10^{-3}	10^{-3}	0,9
2-tert-butyl-4,6-dimethyl-5-HPM	—	10^{-3}	10^{-3}	2,1
2-Amyl-4,6-dimethyl-5-HPM	—	10^{-3}	10^{-3}	2,9
2-Morpholinomethyl-4,6-dimethyl-5-HPM	—	10^{-3}	10^{-3}	4,5

Legend. *Values of K_7 taken from data in [1].

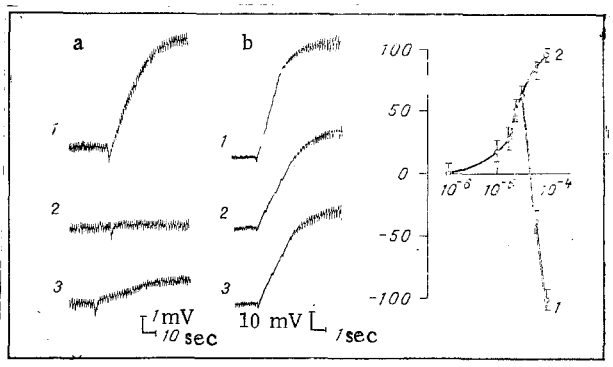

Fig. 1 Fig. 2

Fig. 1. Inhibition of platelet aggregation by indomethacin. A) Low doses of activators: 1) control, 2) arachidonic acid (50 μM), 3) thrombin (0.3 unit/ml) and A23187. Calibration: 1 mV, 10 sec; B) high doses of activators: 1) control, 2) A23187 (1.5 μg/ml), 3) thrombin (1.5 unit/ml). Calibration: 10 mV, 1 sec.

Fig. 2. Dependence of action of ionol on indomethacin-sensitive platelet aggregation on concentration. Abscissa, concentration of ionol (in M); ordinate, activation of aggregation (in %). 1) Ionol; 2) ionol + EDTA (0.5 mM).

solution in ethanol (the ethanol concentration in the incubation medium was 2%) and the samples were preincubated for 5 min at 37°C in medium of the following composition (in mM): Tris-HCl 15, NaCl 134, glucose 5; pH 7.4. Inhibition of aggregation was assessed as the change in its rate relative to the control, expressed as a percentage.

Reagents. Tris-HCl, EDTA, glucose, and 4-methyl-2,6-di-tert-butylphenol were from Sigma, USA; indomethacin and A23187 were from Calbiochem, USA; arachidonic acid was from Serva, West Germany; aspirin was from Lachema, Czechoslovakia; NaCl was from Reakhim, USSR; thrombin was from the Kaunas Medical Preparations Factory, Lithuania. Derivatives of 3-hydroxypyridine (3-HP) and 5-hydroxypyrimidine (5-HPM) were synthesized by the writers previously [1].

EXPERIMENTAL RESULTS

Platelet aggregation *in vitro* can be initiated both with the participation of COG and also independently of this enzyme. The problem of which type of aggregation is involved can be decided by the use of the specific COG inhibitor, indomethacin. If aggregation is inhibited by low concentrations of indomethacin (under 50 μM) this means that the process involves the participation of COG.

To select the conditions for recording GOC-dependent and COG-independent aggregation the action of indomethacin was studied on platelet aggregation induced by different concentrations of A23187, thrombin, and arachidonic acid. It was found that aggregation induced by arachidonic acid in a concentration of 50 μM was completely blocked by indomethacin in a concentrations of 50 μM (Fig. 1). The inhibitory action of indomethacin on platelet aggregation induced by A23187 and thrombin was rather weaker. If aggregation was triggered by high concentrations of A23187 (1.5 μg/ml) and thrombin (1.5 unit/ml), indomethacin in a concentration corresponding to its specific action (50 μM) could not inhibit it. According to data in the literature [4] the existence of indomethacin-sensitive and indomethacin-insensitive aggregation is due to different ways whereby Ca^{++} ions, the secondary messenger in the aggregation process, enter the cytoplasm. In low concentrations of the ionophore, thrombin, or arachidonic acid, Ca channels are formed through the participation of the COG reaction, and accordingly aggregation under these conditions is sensitive to specific inhibitors of COG.

The conditions chosen for recording indomethacin-sensitive and indomethacin-insensitive platelet aggregation enabled us to investigate the action of several antioxidants on aggregation triggered by the two method. It was thus possible to compare their action with the action of indomethacin and to solve the problem of the specificity of their effect.

As antioxidants we used 4-methyl-2,6-di-tert-butylphenol (ionol) and also antioxidants of two new classes: derivatives of 3-HP and of 5-HPM synthesized by ourselves. All the antioxidants tested inhibited indomethacin-insensitive aggregation. Ionol was the most effective of them ($ED_{50} = 3 \times 10^{-5}$ M). Antioxidants of the 3-HP and 5-HPM class had low antiaggregating activity (Table 1). If antioxidants can exert an inhibitory action on the COG-dependent reaction, it might be expected that if aggregation along the indomethacin-sensitive pathway is triggered, their antiaggregating activity would rise significantly, and they would exert their action in lower concentrations. However, this did not happen. The antiaggregating effect of the test compounds on indomethacin-sensitive aggregation did not differ from their effect on indomethacin-insensitive aggregation. On this basis it can be postulated that the antiaggregating activity of the antioxidants studied is unconnected with their action on the COG reaction. It was also found that ionol, in high concentrations, can accelerate indomethacin-sensitive aggregation (Fig. 2). This effect of ionol was blocked by the presence of EDTA in the incubation medium, so that activation of aggregation by ionol can be explained by damage to the plasma membrane and entry of Ca^{++} inside the cell. On stimulation of platelets with high doses of thrombin and A23187 this effect was not observed, probably because the two activators themselves induce powerful inward flows of Ca^{++} inside the cell as well as liberation of Ca^{++} from intracellular depots, and against the background of this sharp rise in the intracellular Ca^{++} concentration correlation was not observed between the antiaggregating activity of the compounds tested and their antiradical properties. It must also be pointed out that an inhibitory effect on platelet aggregation also was demonstrated by the use of 3-dimethylcarbamoylhydroxypyridine and also of 2,4,6-trimethylpyridine, compounds which do not contain exposed OH-groups, i.e., which do not possess antiradical activity. Taken together, these facts suggest that inhibition of aggregation is linked with the direct, nonspecific action of the test compounds on cellular structures.

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