Comparison of the effect of various sympathicolytics on thrombocyte aggregation

SIR—According to O'Brien (1963) the aggregation of thrombocytes induced by adrenaline and noradrenaline is mediated by specific receptors in thrombocytes themselves because phentolamine blocks this aggregation.

There is also a relation between the action of catecholamines on thrombocyte aggregation and their stimulating effect upon the smooth musculature of the nictitating membrane of the cat (O'Brien, 1964). If we assume that thrombocyte receptors possess the properties of adrenergic α-receptors, a contradiction exists in the fact that adrenaline-induced aggregation is also inhibited by dichloroisoprenaline which has good β-receptor blocking properties.

We have now compared quantitatively the inhibitory effect of phentolamine and tolazoline with that of some β-receptor blocking drugs.

In our experiments we used heparinized platelet rich human plasma, prepared according to Born & Cross (1963). We determined the inhibitory effect of sympathicolytics using our own modification of O'Brien's method (Ryšánek, Švehla & others, 1966). As a measure of 100% aggregation we took the drop in extinction in the plasma 2 min after adding either adrenaline in a concentration of 5×10^{-5}M, or ADP in a concentration of 5×10^{-4}M. We exposed thrombocytes to the action of the examined substances for 10 min. We expressed the degree of inhibition in per cent as the reciprocal of aggregation.

The drop in extinction was measured continuously after the addition of adrenaline during 120 sec, and then the samples were centrifuged for 60 sec at 25 rev/min. After centrifugation the drop in extinction was measured once more. We chose the time of 120 sec to be able to observe the first phase of the aggregating effect of adrenaline. The results of the measurement after aggregation proved more suitable for evaluation. The drop in extinction is given in per cent of the original value. For the evaluation of the mode of inhibition we used Lineweaver's & Burk's method (1934).

TABLE 1. INHIBITION OF THROMBOCYTES AGGREGATION BY SYMPATHICOLYTIC DRUGS

Drug	Aggregation induced by adrenaline			Aggregation induced by ADP		
	Conc. M 5×	Inhibition % (± s.d.)	Significance of inhibition P	Conc. M 5×	Inhibition % (± s.d.)	Significance of inhibition P<
Phentolamine	10^{-6}	98·25 (22·19) (8)*	<0·001			
Tolazoline	10^{-6}	95·19 (10·63) (8)	<0·001			
Pronethalol	10^{-4}	69·18 (17·22) (8)	<0·001	10^{-4}	43·55 (25·77) (15)*	<0·001
	10^{-5}	2·35 (11·27) (8)	<0·5	10^{-5}	6·21 (10·98) (8)	<0·2
	10^{-5}	8·32 (12·83) (8)	<0·2			
Isopropylmethoxamine	10^{-4}	67·05 (16·15) (8)	<0·001	10^{-4}	7·68 (8·73) (10)	<0·05
	10^{-5}	13·86 (18·87) (8)	<0·1	10^{-5}	0·24 (4·18) (8)	<0·9
	10^{-6}	1·23 (7·08) (8)	<0·6			
Propranolol	10^{-4}	95·50 (3·72) (9)	<0·001	10^{-4}	92·79 (2·78) (8)	<0·001
	10^{-5}	5·81 (13·58) (10)	<0·3	10^{-5}	−11·11 (19·70) (9)	<0·2

* No. of experiments

From our results (Table 1) it follows that among the investigated compounds phentolamine and tolazoline appeared the most potent, inhibiting adrenaline-induced thrombocyte aggregation 100% even at 5×10^{-6}M. β-Receptor blocking agents, pronethalol, isopropylmethoxamine, and propranolol, had a significant inhibitory effect only in 5×10^{-4}M concentrations. The inhibitory effect of phentolamine and tolazoline appeared when a concentration ten times lower than that of adrenaline was used. On the other hand the β-receptor blocking drugs were active only in a concentration ten times higher than that of adrenaline in the incubation mixture.

β-Receptor blocking agents at 1×10^{-4}M inhibited the second phase of adrenaline-induced aggregation only. These findings by themselves indicate that the inhibitory action of the β-receptor blocking drugs is probably a non-specific one.

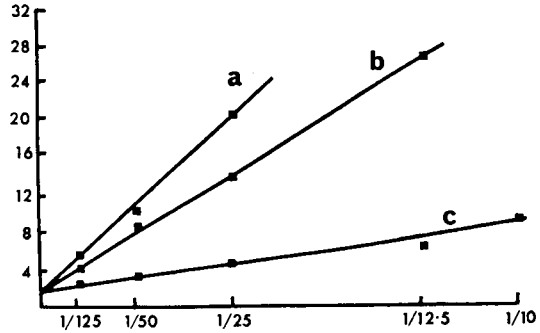

FIG. 1. Interference of phentolamine in different concentrations with adrenaline induced platelet aggregation. Abscissa: Reciprocal values of adrenaline concentration departing from the molar concentration 1×10^{-7}. Ordinate: Reciprocal values of the degree of inhibition expressed in % of drop in extinction. phentolamine concentration, a, 5×10^{-8}M; b, 5×10^{-9} M. c, control group.

The finding of a competitive inhibition of the first phase of adrenaline-induced thrombocyte aggregation by phentolamine (Fig. 1) appears to us acceptable evidence that phentolamine inhibits the adrenaline receptors in thrombocytes. A similar competitive inhibition is produced by tolazoline.

This competitive inhibition was not seen with the β-receptor blocking agents even in high concentrations.

β-Receptor blocking agents also inhibited significantly at 5×10^{-4}M the ADP-induced thrombocyte aggregation. The most potent was propranolol (Table 1). The least effective was isopropylmethoxamine. These drugs accelerated the disaggregation of thrombocytes aggregated by ADP and thus in this sense they resembled desipramine (Mills & Roberts, 1966).

Research Institute of Experimental Therapy,
Prague-Krč,
Budějovická 800,
Czechoslovakia.

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

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