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Effects of Adrenergic Blockers on Platelet Aggregation

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The action of orciprenaline, tolazoline, propanolol and inpea on platelet aggregation induced by ADP epinephrine and norepinephrine was studied *in vitro* in human platelet-rich plasma.

Orciprenaline did not significantly affect aggregation induced by ADP. Tolazoline inhibits the aggregation induced by epinephrine and norepinephrine more intensely than the β-blockers. Inpea blocks the platelet aggregation induced by epinephrine and norepinephrine to a greater extent than propanolol at similar concentrations. The β-blockers inhibit platelet aggregation non-specifically.

Platelet aggregation can be induced by ADP and other agents including thrombin, collagen, 5-HT, epinephrine and norepinephrine (9, 14, 1, 18). Aggregation induced by these substances is mediated through a release of ADP from the platelets (7, 9, 15, 16).

ARDLIE *et al.* (1) suggest that the formation of cAMP in platelets might be involved in the control of platelet aggregation. All drugs that increase cAMP, through the inhibition of phosphodiesterase or the stimulation of adenylcyclase, inhibit platelet aggregation (2, 10, 19, 20).

The papers of MILLS (12, 13) are in agreement with the articles of ARDLIE *et al.* (1) concerning the cAMP in platelets. It is one of the controlling factors in the process of platelet aggregation, but they suggest that platelet aggregation induced by epinephrine is through α-receptors, not through β-receptor mechanisms because the isoproterenol does not enhance or inhibit the aggregation induced by epinephrine and norepinephrine (6, 12).

The object of the present work is to evaluate the effect of the catecholamine on platelet aggregation, comparing the inhibitory potential of an α-blocker (tolazoline) and two α-blockers on platelet aggregation induced by ADP, epinephrine and norepinephrine.

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Materials and Methods

Healthy volunteers between 18 and 23 years of age were used in this study. The blood was collected in centrifuge plastic tubes containing 1/10 volume of 3.8 per cent trisodium citrate. To obtain platelet rich plasma (PRP) the blood was centrifuged for 10 minutes at 100-145 g and at room temperature (15-20° C). The final number of platelets in the experimental samples was 250-300 $\times$ 10^3/ml. After the blood was collected, it was centrifuged —for 30 minutes at 27 000 g to obtain platelets for plasma (PPP).

The aggregation of platelets was measured using a technique based on the turbidimetric method of BORN and CROSS (4, 5). Upchurch aggregometer was employed. The recorder scale of the aggregometer was calibrated with PPP (100 per cent light transmission) and PRP (0 per cent light transmission). Aggregation and inhibition were measured by the change in the optical density of the aliquot PRP.

The drugs used in the experiment were: epinephrine, norepinephrine, and ADP from Sigma; orciprenaline (Boehringer Sohn Ingelheim), propanolol (ICI Farma), inpea (Liade) and tolazoline (Ciba).

0.9 ml of PRP and 0.1 ml of the test solution were added. The incubation time of PRP and test solution was 2 minutes at 37° C.

The results were analysed by the *t* test for each group with the control. All values are shown as means ± SE.

Results

Platelet aggregation induced by epinephrine is more intense than platelet aggregation by norepinephrine at identical concentrations. This inhibition produced by propanolol and tolazoline is proportional to both blocker concentrations (table I).

Orciprenaline inhibits aggregation pro-

Table I. *Effect of propanolol and tolazoline on ADP-induced aggregation in PRP human plasma.*
The response is measured in % of control (ADP).

CONCENTRATION (M)	EFFECT (%)
ADP 3.57×10^{-6}	100
Propanolol	
3.27×10^{-5}	79.62 ± 4.91
3.27×10^{-4}	64.51 ± 2.50
3.27×10^{-3}	43.59 ± 2.17
Tolazoline	
3.25×10^{-5}	83.54 ± 4.33
3.25×10^{-4}	77.73 ± 1.12
3.25×10^{-3}	62.37 ± 5.09

Table II. *Effect of orciprenaline on ADP-induced aggregation in PRP human plasma.*
The response is measured in % of control.

Orciprenaline [M]	ADP		
	(1.17×10^{-6})	(2.34×10^{-6})	(3.57×10^{-6})
Control	100	69.28±5.21	58.87±4.21
2.54×10^{-6}	105.08±7.23	70.89±6.57	58.46±4.47
2.54×10^{-5}	95.10±7.82	67.92±8.12	57.65±5.15
2.54×10^{-4}	89.92±9.04	67.58±7.53	52.71±5.74

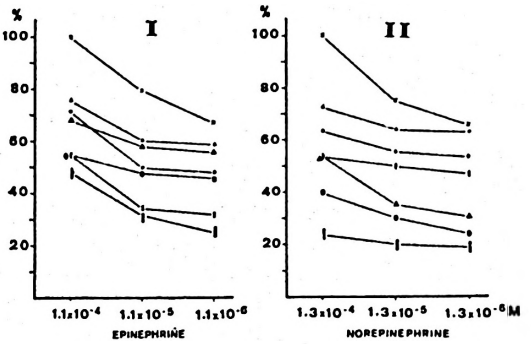

Fig. 1. *Aggregation of platelets induced by epinephrine (I) and norepinephrine (II).* ×—× control: epinephrine (I) or norepinephrine (II), ▲—▲ propanolol (3.27×10^{-5} M), ●—● propanolol (3.27×10^{-4} M), ■—■ propanolol (3.27×10^{-3} M), ▲—▲ tolazoline (3.25×10^{-5} M), ●—● tolazoline (3.25×10^{-4} M), ■—■ tolazoline (3.25×10^{-3} M). Incubation time: 2 minutes.

Table III. *Effect of propanolol and Inpea on the aggregation induced by epinephrine and norepinephrine in PRP human plasma.*
The response is measured in % of control. (Epinephrine and norepinephrine: 1.36×10^{-4} M.)

	Effect %		
	(1.36×10^{-4} M)	(1.36×10^{-5} M)	(1.36×10^{-6} M)
		Epinephrine	
Control	100	76.45 ± 3.47	65.02 ± 5.33
Propanolol 3.27×10^{-3} M	56.10 ± 3.21	40.37 ± 3.88	32.94 ± 5.19
Inpea 2.94×10^{-3} M	47.52 ± 2.85	30.86 ± 4.02	27.07 ± 3.33
		Norepinephrine	
Control	88.40 ± 1.86	65.16 ± 2.17	40.81 ± 2.74
Propanolol 3.27×10^{-3} M	46.33 ± 3.25	35.07 ± 3.74	26.41 ± 4.02
Ipnea 2.94×10^{-3} M	39.46 ± 3.88	26.19 ± 4.16	21.10 ± 3.42

duced by ADP but the differences were not statistically significant (table II). Propanolol and tolazoline block platelet aggregation induced by epinephrine. At the same concentrations inhibition by tolazoline is slightly higher (fig. 1).

Propanolol and tolazoline —at the same concentrations— block platelet aggregation induced by norepinephrine. At the same concentrations tolazoline inhibition is much more intense than propanolol induced.

Inpea blocks platelet aggregation produced by epinephrine and norepinephrine more strongly than propanolol (table III).

Discussion

Platelet aggregation produced by epinephrine is more intense than that obtained with norepinephrine. These results have already been indicated by other authors (11, 15, 18). On the other hand, the inhibition of platelet aggregation produced by ADP is more intense with propanolol than tolazoline. These results appear to indicate a greater importance of β-receptors than α-receptors in the platelet aggregation phenomenon. However orcipreNaline by itself does not modify the platelet aggregation induced by ADP in any significative way, and tolazoline inhibits the platelet aggregation induced by epinephrine or norepinephrine more intensely than propanolol (fig. 1). These results suggest that the platelet aggregation produced for these amines is realized through α-receptors. The fact of propanolol is capable of blocking even strongly the platelet aggregation induced by epinephrine and norepinephrine, does not invalidate the theory that platelet aggregation produced by catecholamines is mediated by α-receptors. The β-blockers would act non-specifically fundamentally affecting the second wave —that is due to the liberation of ADP by platelets—, while the α-blockers would fundamentally inhibit the first phase that would be the consequence of the interaction of these amines with the α-receptors (3, 13).

The results obtained in this paper are in agreement with the above previous facts, that inhibition with propanolol is only more intense than inhibition obtained with tolazoline, only slightly inhibited when the platelet aggregation is induced by ADP, and very inferior (10-100 folds) when it is inhibited by catecholamines.

It is assumed that the inhibition produced by β-blockers would be a non-specific effect of these compounds on the membrane of platelet (7). This effect appears not to be connected to the quinidin-

like activity of propanolol since the inpea, —β-blocker which, unlike propanolol, does not have membrane activity but has intrinsic activity (8)—, inhibits at similar concentrations of propanolol the piatelet aggregation induced by epinephrine and norepinephrine more intensely than propanolol. The fact that the potential β-blocker of propanolol is 25 times greater than that of inpea corroborates the hypothesis that the inhibition of the platelet aggregation induced by these drugs is a non-specific form.

Resumen

Se estudia *in vitro* la acción de la orciprenalina, tolazolina, propanolol e inpea, sobre la agregación plaquetaria inducida por ADP, adrenalina y noradrenalina, utilizando plasma rico en plaquetas.

La orciprenalina afecta ligeramente y de forma no significativa la agregación plaquetaria inducida por ADP. La tolazolina bloquea la agregación inducida por adrenalina y noradrenalina más intensamente que los bloqueadores beta adrenérgicos. El inpea bloquea la agregación plaquetaria inducida por adrenalina y noradrenalina más intensamente que lo hiciera el propanolol a concentraciones semejantes. Los bloqueadores β-adrenérgicos inhiben la agregación plaquetaria de forma no específica.

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