DRUG-INDUCED INHIBITION OF GUINEA PIG PLATELET AGGREGATION UNRELATED TO THEIR β-ADRENOLOYYTIC ACTIONS

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Abstract—Inhibitory actions on adenosine diphosphate (ADP)-induced platelet aggregation of atenolol, dl- and d-D-32, IPS-339, pindolol and propranolol were investigated in guinea pigs for the purpose of obtaining a clue about a possible mechanism for the disaggregatory phenomenon of β-adrenoceptor blocking agents. The effects of verapamil and procaine on guinea pig platelet aggregation were also examined. All of these agents including verapamil and procaine showed a dose-dependent inhibitory effect on platelet aggregation, and their relative potencies determined on the basis of the molar concentrations producing a 50% inhibition of ADP-induced aggregation were in descending order: IPS-339 > propranolol > verapamil > dl-D-32 = d-D-32 > pindolol > procaine > atenolol. This order of relative potencies of the inhibitory actions of these test compounds on platelet aggregation was well correlated to those of local anaesthetic action in guinea pigs (r=0.932, P<0.01) and lipophilicity (r=-0.899, P<0.01), while it did not agree with the orders of potency of β-adrenoceptor blocking action, intrinsic sympathomimetic action and vasodilator action. From these results, it may be reasonable to propose that inhibitory actions of β-adrenoceptor blocking agents and local anaesthetics on platelet aggregation are caused through the same mechanism or through a very similar one.

According to Mills and Roberts (1) and O’Brien (2), the drugs like imipramine and related agents, anti-histamines and local anaesthetics which have a stabilizing action on biological membranes can inhibit the aggregation of human platelets by adenosine diphosphate (ADP). Recently, based on these observations, the inhibitory action of β-adrenoceptor blocking agents on platelet aggregation has been studied by many researchers not in guinea pigs but in rabbits (3) and in humans (4–9). It has been suggested that in human and rabbit platelets, the aggregation can be inhibited by β-adrenoceptor blocking agents in vitro, and this inhibitory effect is usually due not to their β-adrenoceptor blocking actions, but to their direct actions on the platelet membrane (3–6, 8, 9). Most of them, however, have not been fully discussed with regard to the participation of other pharmacological actions unrelated to their β-adrenoceptor blocking actions in the inhibitory mechanism of platelet aggregation caused by the β-adrenoceptor blocking agents. Therefore, we performed the present experiments with guinea pigs to obtain further information concerning the inhibitory phenomenon of β-adrenoceptor blocking agents on ADP-induced platelet aggregation by using several agents which have individually different...
Table 1. Pharmacological features of 6 β-adrenoceptor blocking agents, procaine and verapamil on several parameters listed below

| Test compounds | Inhibitory action on ADP-induced platelet aggregation in guinea pigs | Local anaesthetic action in guinea pigs | Partition coefficient in n-octanol/pH 7.4 Mollvaine’s buffer | β-Adrenoceptor blocking action in rats<sup>a,b</sup> | Intrinsic sympathomimetic action in reserpined rats<sup>a</sup> | Vasodilator action in canine femoral vascular bed<sup>b</sup> |
|----------------|-------------------------------------------------|----------------------------------|-----------------------------------|-----------------------------|----------------------------------|-------------------------|
| IPS-339        | 0.94 (0.69–1.3) × 10<sup>-6</sup> M<sup>a</sup> | 2.2                             | 16.0 (5.7–44.8)                  | 56.0                        | 5                               | 3                       | (−)                     | 4                       |
| Propranolol    | 2.1 (1.6–2.8)                                  | 1                               | 24.2 (11.5–50.8)                 | 2.4                         | 3                               | 4                       | (−)                     | 5                       |
| dl-D-32        | 3.7 (2.7–5.0)                                  | 0.57                            | 36.6 (17.9–75.0)                 | 2.1                         | 2                               | 2                       | (−)                     | 2                       |
| d-D-32         | 3.7 (2.8–4.9)                                  | 0.57                            | 42.0 (23.3–75.6)                 | 0.56                        | (−)                             | (−)                     | (−)                     | 2                       |
| Pindolol       | 11.7 (6.1–23.8)                                | 0.18                            | 178.0 (122.7–258.1)              | 0.16                        | 0.2                             | 1                       | 1                       | (+)                     | 6                       |
| Atenolol       | 36.2 (24.0–53.2)                               | 0.058                           | 1000 (625–1600)                  | 0.025                       | 0.01                            | 4                       | 5                       | (−)                     | 8                       |
| Procaine       | 24.8 (11.3–60.0)                               | 0.078                           | 110.0 (58.5–206.8)               | 0.20                        | 0.5                             | (−)                     | (−)                     | 7                       |
| Verapamil      | 3.0 (2.3–3.8)                                  | 0.70                            | 88.0 (57.5–134.6)                | 0.46                        | 23.0                            | (−)                     | (−)                     | (−)                     | 1                       |

<sup>a</sup> 95% confidence limit.  
<sup>b</sup> The figures indicate the order in their β-adrenoceptor blocking activities: the smaller the number, the stronger the activity.  
<sup>c</sup> Relative potency (R.P.) was expressed as a value relative to that of propranolol which was taken as 1.  
<sup>d</sup> Our unpublished data: β-Adrenoceptor blocking actions of test compounds were determined on the basis of their inhibitory effects on the tachycardia (β<sub>1</sub>) and hypotension (β<sub>2</sub>) induced by i.v. isoproterenol (0.1 μg/kg) in pentobarbionate anaesthetized rats. Intrinsic sympathomimetic action of test compounds was checked by cumulative i.v. injection into pentobarbionate anaesthetized rats which were pretreated with reserpine (1 mg/kg i.p.) at 48 and 24 hr before the experiments, and these rats were vagotomized and adrenalectomized just before the experiments. (−) indicates no or extreme weak effect on individual parameters.
activities or values in β-adrenoceptor blocking action, local anaesthetic action, lipophilicity, intrinsic sympathomimetic action and vasodilator action as listed in Table 1. Furthermore, we checked the local anaesthetic activities in guinea pigs and lipophilicities of the 6 β-adrenoceptor blocking agents used in the present experiments. In addition to these experiments, the properties of a calcium antagonist, verapamil, and a local anaesthetic, procaine, were also investigated in the same experimental system.

Materials and Methods

Platelet aggregation: Blood was drawn from the abdominal aorta of anaesthetized male Hartley guinea pigs (510–980 g) with pentobarbitone sodium (36 mg/kg i.p.) into plastic syringes containing 3.8% sodium citrate (1:9 V/V citrate: whole blood). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were centrifuged at 170×g for 10 min and 1500×g for 15 min at room temperature, respectively. The platelet count of PRP was adjusted withPPP to the range of 2×10^5–5×10^5 platelets/mm³ by using a Coulter counter (Coulter Electronics Inc.). Platelet aggregation was observed by measuring the change in optical density in an aggregometer (Sienco Dual Sample Aggregation Meter, DP-247E, Sienco Co.) at 37°C, according to the method originally developed by Born (10). After the incubation of 0.25 ml PRP with 0.014 ml solution containing test compounds at several concentrations or saline as a control solution for 5 min at 37°C, 0.014 ml of adenosine diphosphate solution (6×10^-5 M) was added to this solution as an aggregating agent. Light transmission was set at 0% with PRP and at 100% with PPP. These results were plotted on a log scale (Fig. 2), and IC50 values (the concentration of the test compounds required for a 50% inhibition of platelet aggregatory response caused by ADP) (Table 1) were obtained for each agent from at least 6 concentration-inhibition curves.

Local anaesthetic action: Local anaesthetic action was measured by intracutaneous wheal in guinea pigs according to the method of Bülbring and Wajda (11). Five minutes after intradermal injection of the test compounds, their local anaesthetic activities were checked on the basis of existence or non-existence of skin reflex at the injection site. ED50 values (the dose of the test compounds required for the disappearance of skin reflex in 50% of animals) were calculated by the Litchfield and Wilcoxon method (12) (Table 1). Five to six experiments were done to obtain each agent’s ED50 value.

Lipophilicity (Partition coefficients): A value for the partition coefficient was obtained by measuring the amount of a test compound that moved into the n-octanol layer from the aqueous one, according to the method of Lombardino et al. (13). Briefly, 30 µmole of each test compound was added to the solution of 15 ml Macilvaine’s buffer (pH 7.4) and 15 ml n-octanol, and the solution was thoroughly mixed by mechanical shaking for 15 min. Then the solution was centrifuged at 2500 rpm for 5 min, and the lower aqueous and upper n-octanol layers were carefully separated. Each solution was directly assayed to measure the amount of a test compound by using an UV spectrophotometer (QV-50, Shimazu Co.). Each value in Table 1 represents the mean of two experiments.

The following agents used in the present study were: adenosine diphosphate (P-L Biochemicals Inc.), dl-atenolol hydrochloride (ICI), d- and dl-D-32 [d- and dl-1-tert-butylamino-3-(2',3'-dimethylphenoxy)-2-propanol hydrochloride] (Teikoku Hormone Pharmaceutical Co.,) IPS-339 [dl-1-tert-butylamino-3-(9-fluorenylideneaminoxy)-2-propanol hydrochloride], dl-pindolol maleate
procaine hydrochloride (Sankodo), dl-propranolol hydrochloride (ICI) and verapamil hydrochloride (Knoll AG). Unless otherwise stated in the text, β-adrenoceptor blocking agents were used in their racemic forms.

Each mean value is given with a standard error and differences between mean values were compared by the Student's t-test, and those with a p value of 0.05 or less were considered significant.

Results

Effects of test compounds on platelet aggregation: Platelet aggregation produced by adenosine diphosphate (ADP) in the present experiments using the blood taken out from guinea pigs was reproducible and did not change with time for at least 4 hr. As can be seen in Fig. 1, the light transmission curve of platelet aggregation induced by 3 μM ADP was composed of two phases, as was observed by MacMillan (14) and Mills and Roberts (1). Only the secondary phase of aggregation was preferentially inhibited at the lower concentration of any of the test compounds. The primary phase aggregation, however, was also inhibited with relatively higher concentrations of the test compounds. The decrease of light transmission and oscillation exerted by adding these compounds except for procaine and the depression of shape change after subsequent addition of ADP were concomitantly observed in our experimental system (Fig. 1). ADP-induced aggregation was inhibited by all test compounds used in our study within the range of 10^{-5} to 10^{-2} M in a concentration dependent manner (Figs. 1 and 2). As shown in Table 1, IC50 values of these compounds were 9.4×10^{-5}, 2.1×10^{-4}, 3.7×10^{-4}, 1.2×10^{-3}, 3.6×10^{-3}, 2.5×10^{-3} and 3.0×10^{-4} M for IPS-339, propranolol, dl- and d-D-32, pindolol, atenolol, procaine and verapamil, respectively. To clearly compare the relative potencies of the test compounds, IC50 values were expressed as values relative to that of propranolol which was taken as 1; and they were approximately 2.2 for IPS-339, 0.6 for dl- and d-D-32, 0.2

![Fig. 1. Platelet aggregation by adenosine diphosphate 3×10^{-6} M after preincubation (5 min) with propranolol (Prop) atenolol (Ate), IPS-339 (IPS), dl- and d-D-32, procaine (Proc) and verapamil (Ver).](image-url)
for pindolol, 0.06 for atenolol, 0.08 for procaine and 0.7 for verapamil. Therefore, the order of inhibitory potency was as follows: IPS-339 > propranolol > verapamil > d/-D-32 > pindolol > procaine > atenolol. 

Local anaesthetic action of the test compounds: IPS-339 was the most active agent among the test compounds used in the present study in depressing the skin reflex against the pin-pricks in guinea pigs, followed by propranolol and d and d/-D-32 (Table 1). IPS-339 was approximately 10 times more active than procaine. On the other hand, atenolol showed the lowest activity and was about 8 times less potent than procaine. Pindolol and verapamil were intermediate, as tabulated in Table 1. 

Lipophilicity of the test compounds: Table 1 shows the lipid solubility of the test compounds. The values of the partition coefficient (log P) of the compounds tested ranged from 0.01 for atenolol to 56 for IPS-339.

Discussion

Based on the information obtained in the present experiments with guinea pig platelets, the correlation between the inhibitory action of β-adrenoceptor blocking agents on platelet aggregation and their other pharmacological properties as listed in Table 1 should be discussed here. As shown in Table 1, β-adrenoceptor blocking activities of the test compounds were in the following order: pindolol > d/-D-32 > propranolol > atenolol > IPS-339 (for β1-adrenoceptors) and pindolol > d/-D-32 > IPS-339 > propranolol > atenolol (for β2-adrenoceptors). On the other hand, the potency series for the inhibitory action on ADP-induced aggregation of guinea pig platelets was IPS-339 > propranolol > d/-D-32 and d/-D-32 > pindolol > atenolol > procaine > verapamil. Thus, the order of β-adrenoceptor blocking action was completely different from that of the inhibitory action on platelet aggregation. Although the β-adrenoceptor blocking action of d-D-32 ([α]D25°+25.0°) was very weak (15), and its activity was about 60 times less potent than that of d/-D-32 (unpublished data), the inhibitory activity of d-D-32 on platelet aggregation was equal to that of d/-D-32 (IC50: 3.7×10⁻⁴ M); and the concentration-response curve of d-D-32 in inhibiting ADP-induced aggregation almost completely fitted that of d/-D-32 (Fig. 2 and Table 1). These facts seemed to confirm the concept that the β-adrenoceptor blocking property per se of drugs is not responsible for their inhibitory action on platelet aggregation, and in this respect, our results are compatible with those reported by Bygdeman and Johnsen (16) and Frishman et al. (5).

The agents used in this study, except for pindolol, have no intrinsic sympathomimetic action (ISA) (Table 1), and pindolol showed only a weak anti-aggregatory action compared with other β-adrenoceptor blocking agents without ISA (Fig. 2 and Table 1), suggesting that ISA is not necessary for producing an inhibitory action on platelet aggregation in guinea pigs. This was further
supported by our observations that guinea pig platelets, unlike human ones (9, 17), responded to neither catecholamines nor salbutamol; and furthermore, unlike in human platelets, \( \beta_2 \)-adrenoceptors may not have any role in causing disaggregation in guinea pig platelets (data not included). Very recently, Hansen et al. (18) have shown that non-selective \( \beta \)-adrenoceptor blocking agents increased platelet aggregability by blocking \( \beta_2 \)-adrenoceptors in human platelets and thus may produce a potential hazard in the treatment of cardiovascular disease. In our acute experiments with guinea pig platelets, whether at relatively low concentrations or at higher ones, all \( \beta \)-adrenoceptor blocking agents including pindolol used in the present study did not increase but decreased the light transmission before adding an aggregating agent (Fig. 1).

There are several lines of evidence that many \( \beta \)-adrenoceptor blocking agents also possess such a calcium antagonistic property as to block certain steps of \( \text{Ca}^{++} \) ions movement from extracellular sites to ones of intracellular contractile proteins (19–22). In cardiovascular experiments, practically all \( \beta \)-adrenoceptor blocking agents have been reported to cause a distinct vasodilation and negative chronotropic and inotropic actions, and with high doses, they cause cardiac standstill, just like verapamil or nifedipine (23–27). In addition, it is well known that local anaesthetics block both the generation and the conduction of the nerve impulse by preventing the increase in the permeability of the membrane to \( \text{Na}^{+} \) ions (28). Furthermore, Josephson and Sperelakis (29) and Feinstein et al. (30) have reported that local anaesthetic agents also have an ability to depress the slow inward \( \text{Ca}^{++} \) current and to prevent the mobilization of internally pooled \( \text{Ca}^{++} \) ions. It has also been accepted that the influx of \( \text{Ca}^{++} \) ions from the external medium and the movements of intracellular \( \text{Ca}^{++} \) ions play an important role in the initiation of platelet aggregation and the secretion of various chemical mediators from the platelet storage granules (19). In the present study, the relative potencies of the test compounds including verapamil for causing anti-aggregation roughly paralleled those of their local anaesthetic action (Table 1), and there was a good correlation (\( r=0.932, P<0.01 \)) between the inhibitory action of these compounds on ADP-induced platelet

![Image](image_url)

Fig. 3. Correlation between inhibition of aggregation and inhibition of skin reflex (left panel) and value of the partition coefficient (right panel). Values of 50% inhibition (molar basis) in anti-aggregatory action and local anaesthetic action or values of the partition coefficient were plotted logarithmically. Abbreviations are as in Fig. 2. Confidence band (dotted area) is for 0.95.
aggregation and their local anaesthetic action
(Fig. 3). Based on the previous reports (20–22, 24, 29, 30) and our present results
obtained from guinea pig platelets, it is
reasonable to suggest that the inhibitory
action exerted by β-adrenoceptor blocking
agents on ADP-induced platelet aggregation
in guinea pigs may be due to their properties
to inhibit certain step(s) of the mobilization
of Ca** ions which are commonly shared
with local anaesthetics. Weksler and Pink
(8) have examined the effects of d- and l-
propranolol and practolol on ADP-, epine-
phrine-, collagen- or thrombin-induced
platelet aggregation in humans and reached
almost the same conclusion proposed by us.

It is interesting that a Ca**-antagonist,
verapamil, showed not only an inhibitory
action on platelet aggregation as observed
by Ono and Kimura (31), but also showed a
distinct local anaesthetic action. Its local
anaesthetic activity was approximately two
times more potent than that of procaine.
This may suggest that a generation of the
action potential at the site involved in local
anaesthetic action is at least in part modified
by the change in influx of Ca** ions.

In addition to the well known local
anaesthetic activity, many β-adrenoceptor
blocking agents have also been shown to
possess distinct vasodilating effects (23, 25–27).
However, the relative order of the
vasodilator potency of the test compounds
observed in canine femoral vascular bed,
unlike that of local anaesthetic activity, was
clearly different from that of their anti-
aggregatory potencies (Table 1). When we
take it into consideration that the secondary
phase of platelet aggregation was much more
susceptible to β-adrenoceptor blocking
agents, procaine and verapamil, as mentioned
in the Results, these two incompatible
observations may lead to the following
presumptions: (A). The vasodilating action of
β-adrenoceptor blocking agents is likely due
to their properties of inhibition of Ca** ion
transport, probably inhibition of Ca** move-
ment via cell membranes of blood vessels,
like that of Ca**-antagonists. On the other
hand, (B), their anti-aggregatory activities
are in a greater part due to the inhibition of
mobilization of internally sequestered or
stored Ca** ions.

As clearly seen in Fig. 3 and Table 1, there
was a good negative correlation (\(r=-0.899, 
P<0.01\)) between the IC50 values (the
concentration required for 50% inhibition) of
anti-aggregatory activity of the test com-
ounds and their partition coefficients. Thus,
the greater the lipophilicity of the com-
ounds, the greater their ability to inhibit the
platelet aggregation and also the stronger
the local anaesthetic action.

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