Competitive inhibition of platelet thromboxane A2 receptor binding by picotamide.

REPRINTED FROM:

European Journal of Pharmacology

Volume 169, No. 1, 4 October 1989

Competitive inhibition of platelet thromboxane A_2 receptor binding by picotamide

Pietro A. Modesti, Andrea Colella, Rosanna Abbate, GianFranco Gensini and GianGastone Neri Serneri

Clinica Medica I, University of Florence, Florence, Italy

pp. 85–93

ELSEVIER SCIENCE PUBLISHERS B.V.— AMSTERDAM

European Journal of Pharmacology, 169 (1989) 85–93
Elsevier

EJP 50979

Competitive inhibition of platelet thromboxane A_2 receptor binding by picotamide

Pietro A. Modesti, Andrea Colella, Rosanna Abbate, GianFranco Gensini and GianGastone Neri Serneri

Clinica Medica I, University of Florence, Florence, Italy

Received 16 January 1989, revised MS received 3 July 1989, accepted 11 July 1989

On the basis of indirect pharmacological evidence, picotamide, a methoxy derivative of 4-hydroxy-isophthalic acid (N,N′bis(3-picolyl)-4-methoxy-isophthalamide) has been postulated to inhibit platelet aggregation by competitively interfering with the thromboxane A_2 (TxA_2) platelet receptor. In the present study the interaction between picotamide and TxA_2 receptors on human platelets was investigated by a direct radioligand assay method with [^{125}I]PTA-OH and [^{3}H]U46619 as labelled radioligands. The ONO11120 and U46619 inhibitory constants (K_i) for [^{125}I]PTA-OH binding were 19 ± 4 and 17 ± 3 nM, respectively. Picotamide displaced [^{125}I]PTA-OH binding with a K_i of 1472 ± 321 nM. The K_i for ONO 11120 and U46619 on [^{3}H]U46619 binding were 42 ± 12 and 16 ± 5 nM, respectively, whereas the K_i for picotamide was 1648 ± 431 nM. These data provide evidence that picotamide can directly inhibit the TxA_2 platelet receptor.

Platelets; Thromboxane A_2; Prostaglandins; (Receptors)

1. Introduction

Picotamide, a methoxy derivative of 4-hydroxy-isophthalic acid (N,N′bis(3-picolyl)-4-methoxy-isophthalamide) (Orzalesi et al., 1975), has anti-aggregating properties (De Cunto et al., 1983; Berrettini et al., 1983; Violi et al., 1988) but its mechanism of action is not yet known. The molecular structure of picotamide seems to be critical for the anti-aggregating activity because its chemical precursors (such as 4-OH-isophthalic acid and the 4-OCH_3-isophthalic acid) have been reported to have pro-aggregating effects (De Cunto et al., 1983). On the basis of indirect pharmacological evidence, picotamide has been postulated to inhibit platelet aggregation by competitively interfering with the thromboxane A_2 (TxA_2) platelet receptor (De Cunto et al., 1983; Berrettini et al., 1983; Deckmyn et al., 1985). More recently, picotamide has been suggested to inhibit TxA_2 synthesis (Violi et al., 1988).

The aim of the present study was to investigate the interaction between picotamide and TxA_2 receptors on human platelets by using a direct radioligand assay.

2. Materials and methods

2.1. Materials

ONO 11120 (9,11-dimethylmethane-11,12-methane-16-phenyl-13,14-dihydro-13-aza-15-tetranor-TxA_2) (6,7) was a gift from Dr. Narumiya (Kyoto, Japan). [^{125}I]PTA-OH (2000 Ci/mmol) (9,11-dimethylmethane-11,12-methane-16-(3[^{125}I]-

Correspondence to: P.A. Modesti, Clinica Medica I, University of Florence, 50134 Florence, Italy.

0014-2999/89/$03.50

4-hydroxyphenyl)-13,14-dihydro-13-aza-15-tetra-nor-TxA_2), the labelled hydroxylated form of ONO11120, was obtained from Amersham (Buckinghamshire, Great Britain). U46619 (9,11-dideoxy-11-α,9-α-epoxymethane-prostaglandin $F_{2\alpha}$) was obtained from Sigma Chemicals (St. Louis, MO, U.S.A.) and [^{3}H]U46619 (22.4 Ci/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.). Prostacyclin (PGI_2) was obtained from Upjohn (Kalamazoo, MI, U.S.A.). Picotamide (N,N′bis(3-picolyl)-4-methoxy-isophthalamide) (batch No. 870311) was provided by Samil Inc.-Sandoz Group, Rome, Italy. All the other reagents were obtained from Merck (Darmstadt, F.R.G.) and were of analytical grade.

2.2. Blood sampling and platelet isolation

Blood was withdrawn by venipuncture from 10 healthy volunteers (aged 44-62 years) who had fasted overnight, between 8:00-9:00 a.m., and 15% (v/v) acid citrate dextrose (NIH formula A) was added to prevent coagulation. None of the volunteers had taken any drug for at least 15 days preceding the study. Platelet-rich plasma was prepared as described previously (Neri Serneri et al., 1984). The platelet-rich plasma, adjusted to pH 6.5 with acid citrate dextrose, was centrifuged at $1800 \times g$ for 30 min at 20°C. Platelets were then resuspended in 10 ml of phosphate buffer pH 7.2 (mM: 8 Na_2HPO_4, 2 NaH_2PO_4, 10 KCl, 135 NaCl) and again recentrifuged at $1800 \times g$ for 30 min at 20°C. The supernatant was discarded and the platelets were resuspended in assay buffer containing (mM) 138 NaCl, 5 $MgCl_2$, 1 EGTA and 25 Tris/HCl, pH 7.5. If necessary assay buffer was added to obtain a platelet concentration of 10^6 platelets/μl.

2.3. Binding studies

2.3.1. Kinetics of [^{125}I]PTA-OH binding

The binding of 0.5 nM [^{125}I]PTA-OH (final concentration, f.c.) to 10^8 platelets (in a final volume of 200 μl) was evaluated at 20°C at selected times (1, 2, 3, 5, 8, 10, 12, 20, 30, 40, 50 min). Non-specific binding was assessed at each time by adding 20 μl of ONO11120 (20 μM f.c.). Specific binding at each time was then calculated as the difference between the total and non-specific platelet bound radioactivity. The reversibility of binding was assessed after 10 min by adding a large amount of unlabelled ligand (20 μl of ONO11120, 20 μM f.c.) to the reaction mixture. The residual binding was evaluated at selected times (1, 2, 3, 5, 8, 10, 12, 20, 30 min).

2.3.2. [^{125}I]PTA-OH binding isotherms

The linearity of the specific binding of [^{125}I] PTA-OH with increasing platelet counts in the incubation medium was first evaluated by incubating 0.05 nM [^{125}I]PTA-OH with different final platelet concentrations, ranging between 5×10^4 and 10^6 platelets/μl. The samples were processed as described below.

Platelet suspension (0.1 ml) was incubated in a final volume of 0.2 ml with 0.05 nM (f.c.) [^{125}I]PTA-OH (2000 Ci/mmol) and increasing concentrations of ONO11120 ($0\text{-}4 \times 10^{-6}$ M) for 10 min at room temperature. The residual radioactivity after the addition of ONO11120 (2×10^{-5} M (f.c.) was considered as non-specific binding. Non-specific binding of 0.05 nM [^{125}I]PTA-OH amounted to 25-35% of the total radioactivity bound. After a 10 min incubation, four 4 ml aliquots of ice-cold buffer were added to each tube to stop the reaction and the tube contents were rapidly filtered under reduced pressure through Whatman GF/C glass microfiber filters. Previous time course experiments showed that binding equilibrium is reached under these conditions. The entire washing procedure was completed within about 15 s. The filters were dried under air and counted in a Beckman gamma counter with an overall efficiency of 50%. The experiments were carried out in triplicate and a displacement curve was obtained for platelets from each subject.

2.3.3. Displacement of the [^{125}I]PTA-OH binding

Displacement curves for U46619, PGI_2 and picotamide on [^{125}I]PTA-OH binding to platelets were obtained by adding increasing concentrations of U46619 ($0\text{-}2 \times 10^{-6}$ M), PGI_2 ($0\text{-}5 \times 10^{-4}$ M) and picotamide ($0\text{-}5 \times 10^{-4}$ M) to the incuba-

tion mixture composed of platelets and [^{125}I]PTA-OH (0.05 nM). After 10 min incubation at room temperature, the samples were processed as described above.

2.3.4. Displacement of the [^{3}H]U46619 binding

To study U46619 binding to platelets, the platelet suspension was incubated, in the same volume and under the above-mentioned conditions, with 20 μl of [^{3}H]U46619 (5 nM f.c.). Increasing concentrations (0-4 × 10^{-6} M) of unlabelled U46619 were added to the incubation mixtures. After 10 min incubation at room temperature, four 4 ml aliquots of ice-cold buffer were added to each tube to stop the reaction and the samples were processed as described previously. Preliminary time course experiments showed that, under these experimental conditions, the specific binding reached equilibrium after about 8 min (n = 4).

The residual radioactivity after the addition of 2×10^{-5} M unlabelled U46619 (f.c.) was considered as non-specific binding. The filters were dried under air, suspended in a liquid scintillation solution (Aquasol, NEN, Dreieich, F.R.G.) and counted in a liquid scintillation spectrometer (Packard, LS 1800, Irvine, CA, U.S.A.) with an overall efficiency of 40%.

Displacement curves of the binding of [^{3}H] U46619 to platelets were obtained for ONO11120 (0-2 × 10^{-6} M), PGI_2 (0-5 × 10^{-4} M) and picotamide (0-5 × 10^{-4} M).

2.4. Analysis of data

2.4.1. Kinetic studies

The kinetic constants were calculated from time course experiments according to Weiland and Molinoff (1981). Briefly, the observed association constant (K_{obs}) was calculated as the slope of the pseudo-first order plot ln(LReq)/((LReq) − (LRt)) versus time (t). LReq and LRt indicate the concentration of ligand-receptor complex at equilibrium and at each time (t), respectively. The dissociation rate constant k_{-1} is a first order rate constant because the rate was determined after elimination of the forward reaction following prior incubation of ligand and receptor. The first order integrated rate equation for dissociation is: $\ln(LRt)/(LR) = -k_{-1}t$. LR and LRt are the concentrations of ligand-receptor complex prior to the addition of competing ligand (time 0) and at time t, respectively. The association constant (K_{+1}) was then calculated by the equation: $k_{-1} = (K_{obs} - k_{-1})/[L]$. The kinetically determined dissociation constant (K_d) is given by: $K_d = k_{-1}/k_{+1}$.

2.4.2. Binding isotherms

The total binding at each concentration of the displacement curve at equilibrium was determined by dividing the decay per minute (dpm) of each platelet pellet by the calculated specific activity in dpm/mol (Modesti et al., 1985). These binding isotherms were analysed according to Scatchard (1949).

2.4.3. Determination of antagonist binding affinities

The inhibitory constant for ONO11120, U46619 and picotamide on [^{125}I]PTA-OH and [^{3}H]U46619 binding to platelets was calculated according to Cheng and Prusoff (1973).

Briefly, the inhibitory concentration 50 (IC_{50}) was determined earlier from indirect Hill plots (logit-log plot) by plotting log ((LRi)/(LR) − (LRi)) versus log(i), where LR and LRi are the ligand-receptor concentration in the absence and in the presence of the inhibitor, respectively, and (i) is the inhibitor concentration (0-2000 nM for U46619 and ONO11120, 0-500.000 nM for picotamide). The slope of the plot is −n (Hill coefficient) and the abscissa intercept is equal to the IC_{50}.

The K_i values were then calculated from the relationship: $K_i = IC_{50}/(1 + F/K_d)$, where F and K_d are the free concentration and the dissociation constant of the labelled compound, respectively, the latter being calculated independently from the binding isotherms.

2.4.4. Statistical analysis

Unless indicated otherwise, all data given in the text are means ± S.D. of n experiments. Each experiment was performed in triplicate.

3. Results

3.1. Time course of [^{125}I]PTA-OH binding

[^{125}I]PTA-OH bound rapidly to intact human platelets. The time course experiments revealed a biphasic pattern: the first phase was characterized by a rapid rate of association and equilibrium was reached within about 10 min (fig. 1); in the second phase the total radioactivity bound rose slowly and regularly. Assessment of the time course for the non-specific binding showed a slow, regular increase in the non-displaceable radioactivity during the slow, linear phase of the binding, with a rate of 3.44 fmol/10^8 platelets per hour (n = 5) (fig. 1).

In contrast, the specific binding, obtained by subtracting the non-displaceable binding from the corresponding total binding at each time, reached equilibrium after about 10 min and remained stable at that level during the following 50 min. A rate constant (K_{obs}) of 0.340 min^{-1} for specific binding was observed (the equation for the line was $y = -0.026 + 0.340x$; n = 5) (fig. 2B).

The dissociation curve, measured after 10 min of incubation (fig. 2A), showed a rapid displacement of about 65-75% of the total radioactivity bound. Analysis of the first order rate of dissociation showed a linear pattern, with a k_{-1} of 0.324 min^{-1} (n = 5) (fig. 2C).

The association rate constant (k_1) was 0.032 nM^{-1} min^{-1} and the resulting, kinetically determined dissociation constant K_d was 10 nM.

3.2. Binding isotherms of the [^{125}I]PTA-OH binding

Preliminary evaluation of the specific binding of [^{125}I]PTA-OH (0.05 nM) and [^{3}H]U46619 (5 nM) showed that binding increased linearly with increasing numbers of platelets in the incubation medium.

When platelets were incubated with increasing concentrations of [^{125}I]PTA-OH, saturation of binding was observed at a ligand concentration of about 100 nM (fig. 3). Scatchard analysis of this binding yielded a straight line, indicating a single class of binding sites for [^{125}I]PTA-OH (fig. 3). The equilibrium dissociation constant (K_d) and the maximum concentration of binding sites (B_{max}) determined by Scatchard analysis were 21.5 ± 7.2 nM and 198 ± 63 fmol/10^8 platelets (1194 ± 379 binding sites per platelet, n = 12), respectively. The Hill coefficient (n Hill) for the binding was 0.942 ± 0.099, suggesting the existence of a homogeneous, individual class of binding sites without

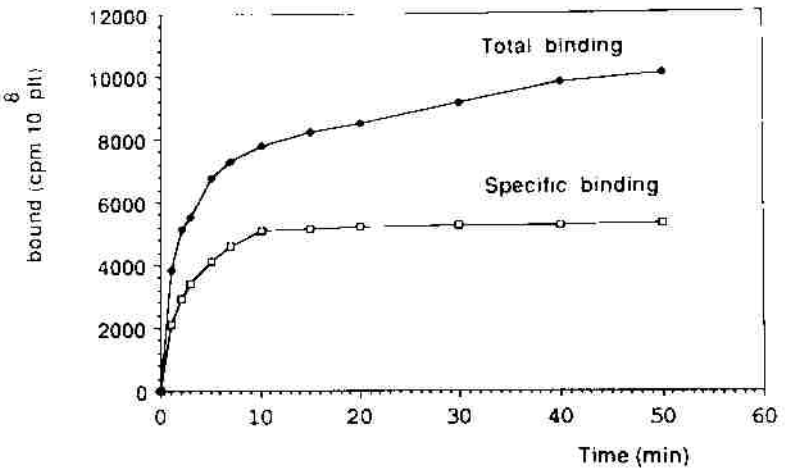

Fig. 1. Time course for the association phase of the binding of [^{125}I]PTA-OH (0.5 nM) to washed human platelets at 20 °C. Platelets (10^8) were incubated with 0.5 nM [^{125}I]PTA-OH (f.c.), and specific and non-specific binding were determined at the time intervals indicated. Specific binding was considered as the radioactivity displaced by 20 μM ONO11120. The points represent the means of triplicate determinations in at least five independent experiments (◆, total binding, □, specific binding).

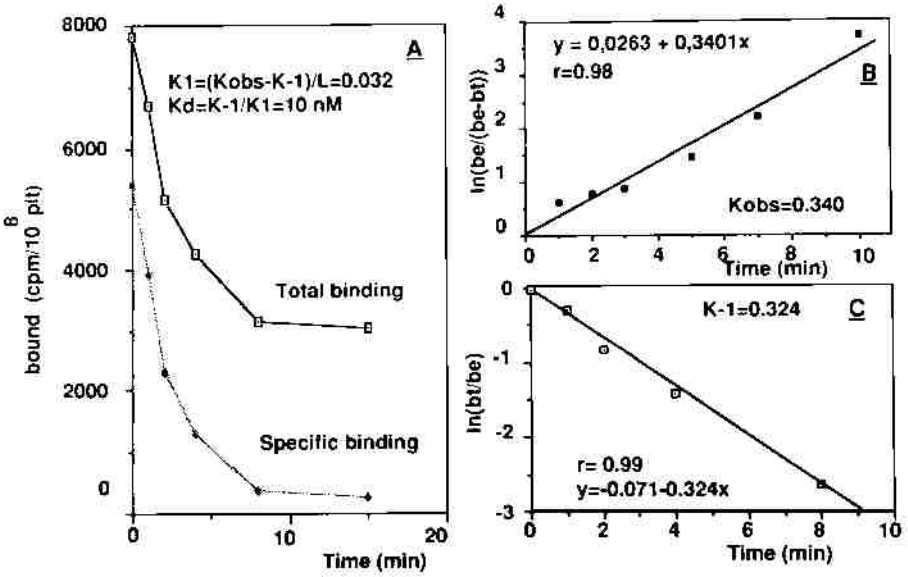

Fig. 2. (A) Time course for the dissociation phase of the specific binding of [^{125}I]PTA-OH (0.5 nM) to 10^8 washed human platelets at 20 °C (n = 5). Dissociation was obtained after 10 min incubation by the addition of 20 μM ONO11120 to the incubation mixture. (B) Specific binding association is plotted according to the pseudo-first order rate equation (K_{obs} = 0.340 min^{-1}). (C) Dissociation is plotted as a first order reaction (k_{-1} = 0.324 min^{-1}; $k_1 = (K_{obs} - k_{-1})/L$ = 0.032 nM^{-1} min^{-1}; $K_d = k_{-1}/k_1$ = 10 nM).

cooperativity. The binding of [^{3}H]U46619 was saturable (fig. 4). The K_d and the B_{max} determined by Scatchard analysis, gave values of 23.5 ± 8.1 nM and 239 ± 92 fmol/10^8 platelets (1439 ± 554 binding sites per platelet, n = 9), respectively (fig. 4).

3.3. Binding affinities of picotamide and other analogues

In competitive binding studies, ONO11120 and U46619 were very effective in displacing [^{125}I]

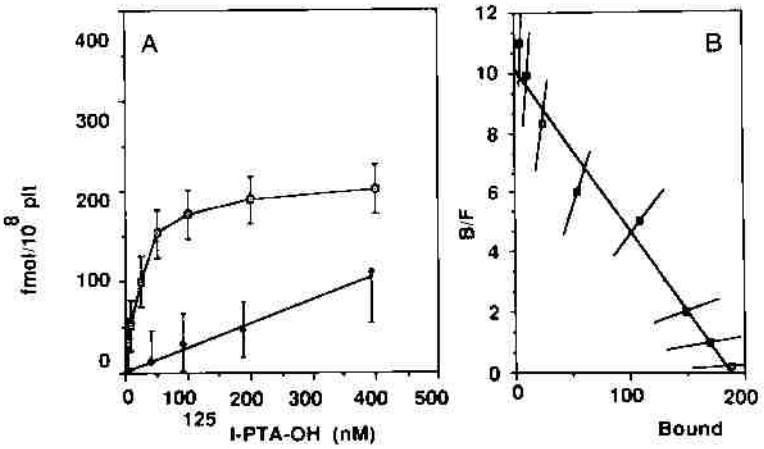

Fig. 3. (A) Saturation curve for [^{125}I]PTA-OH binding to washed human platelets at 20 °C (○, total binding; □, specific binding; ●, non-specific binding) (n = 12). (B) Scatchard analysis of the specific binding (B_{max} = 198 ± 63 fmol/10^8 platelets, K_d = 21.5 ± 7.2 nM).

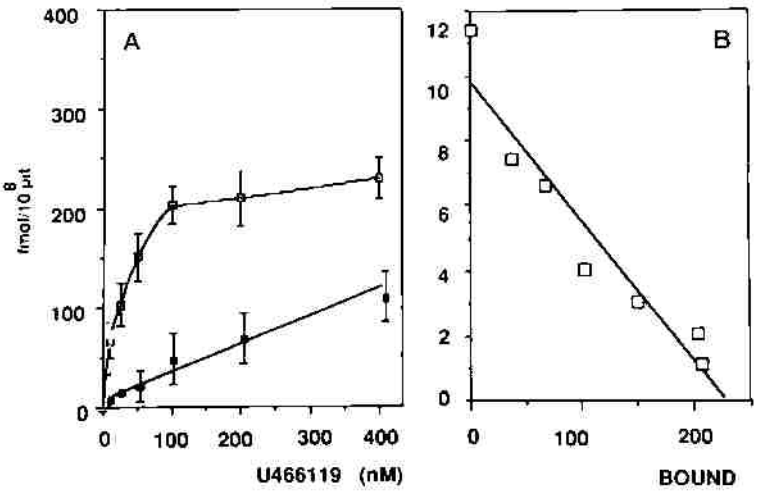

Fig. 4. (A) Saturation curve for [^{3}H]U46619 binding to washed human platelets at 20 °C. Platelets were incubated with [^{3}H]U46619 (5 nM, 22.4 Ci/mmol) plus unlabelled U46619 (0-20 μM) for 10 min at 20 °C. The total binding for each concentration was determined by dividing the decay per minute (dpm) by the calculated specific activity in dpm/mol. Specific binding accounted for 50-60% of the total binding. Each point represents the mean ± S.D. of nine independent experiments (○, total binding; □, specific binding; ■, non-specific binding) (n = 9). (B) Scatchard analysis of the specific binding (B_{max} = 239 ± 92 fmol/10^8 platelets, K_d = 23.5 ± 8.1 nM).

PTA-OH binding. The inhibitory constant (K_i) for ONO11120 and U46619 was 19 ± 4 (n = 10) and 17 ± 3 nM (n Hill = 0.943 ± 0.096, n = 10), respectively. Picotamide displaced [^{125}I]PTA-OH binding with a K_i of 1472 ± 321 nM (n Hill = 0.970 ± 0.075, n = 9) (fig. 5). On the contrary, PGI_2 did not significantly inhibit [^{125}I]PTA-OH binding to platelets.

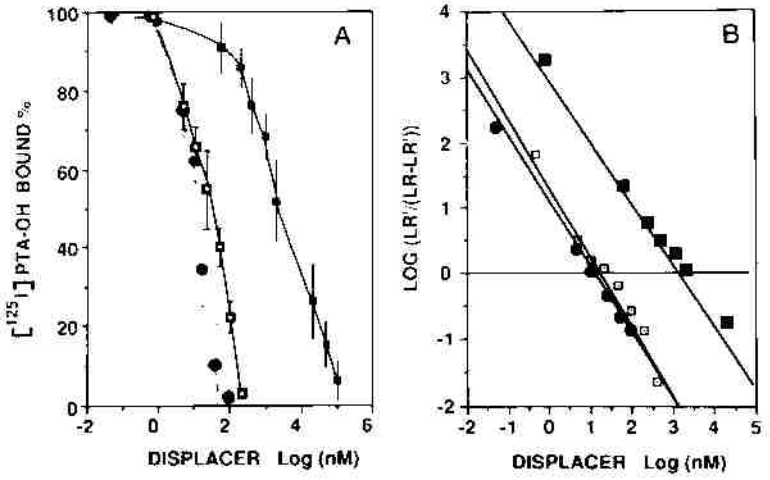

Fig. 5. (A) Displacement of specific [^{125}I]PTA-OH binding by increasing concentrations of ONO11120 (□), U46619 (●) and picotamide (■). Data points represent the means of triplicate determinations in at least 10 independent experiments. (B) Hill plot of the displacement curves. K_i values, calculated according to Cheng and Prusoff (1973), were 19 ± 4 nM for ONO11120 (n = 10, n Hill = 0.942 ± 0.096), 17 ± 3 nM for U46619 (n Hill = 0.943 ± 0.096, n = 10) and 1472 ± 321 nM for picotamide (n Hill = 0.970 ± 0.075, n = 9).

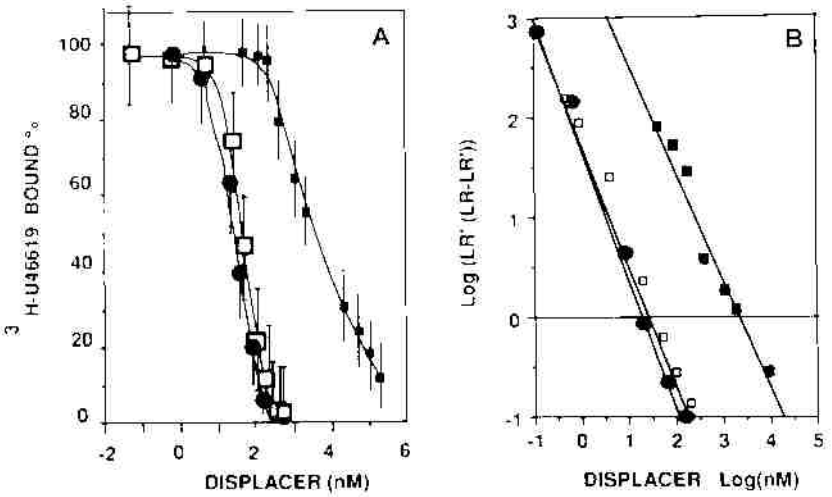

Fig. 6. (A) Displacement of specific [^{3}H]U46619 binding by increasing concentrations of ONO11120 (□), U46619 (●) and picotamide (■). (B) Hill plot of the displacement curves. K_i values, calculated according to Cheng and Prusoff (1973), were 42 ± 12 nM for ONO11120 (n Hill = 1.280 ± 0.042, n = 10), 16 ± 5 nM for U46619 (n Hill = 1.220 ± 0.042, n = 9) and 1648 ± 431 nM for picotamide (n Hill = 1.141 ± 0.061, n = 10).

The inhibitory potencies of ONO11120, U46619 and picotamide on [^{3}H]U46619 binding were also evaluated. The K_is were 42 ± 12 nM for ONO11120 (n Hill = 1.280 ± 0.042, n = 10), 16 ± 5 nM for U46619 (n Hill = 1.220 ± 0.042, n = 9) and 1648 ± 431 nM for picotamide (n Hill = 1.141 ± 0.061, n = 10) (fig. 6). In contrast, PGI_2 did not significantly inhibit [^{3}H]U46619 binding to platelets.

4. Discussion

These binding studies indicate that [^{125}I]PTA-OH effectively binds to an individual class of specific platelet receptors. In fact, [^{125}I]PTA-OH binding fulfilled the criteria of binding to specific receptors, such as saturability, rapidity, reversibility, and specificity of the binding. Moreover, binding was specific for thromboxane receptors because competitive binding experiments showed that the two labelled stable TxA_2 analogues used, [^{3}H]U46619 and [^{125}I]PTA-OH, were displaced by low concentrations of the two unlabelled compounds, U46619 and ONO11120. On the contrary, PGI_2, which is known to bind to different specific receptors, did not displace the two TxA_2 analogues to any extent.

Platelet TxA_2 receptors have been investigated with different stable TxA_2 analogues. Two different classes of receptors have been reported by Hung et al. (1983) using 13-azaprostanoic acid. Armstrong et al. (1983) found two different binding sites for the two epimers, U46619 and U44069, but only the receptor for the active form was postulated to be physiological. The presence of two classes of TxA_2 receptors, a high-affinity class (the putative physiological receptor) and a low-affinity class, has been challenged by subsequent investigations (Mais et al., 1985a; Narumiya et al., 1986; Kattelman et al., 1986; Liel et al., 1987; Hanasaki and Arita, 1988) that have provided convincing evidence for existence of a single class of specific binding site for TxA_2 on platelets.

In the present study, the dissociation constants for [^{125}I]PTA-OH binding calculated with three different complementary methods (kinetic studies, saturation curves and competitive binding studies) were of the same order of magnitude (low nanomolar range), thus indicating the presence of a single class of specific receptors. The number of binding sites we found was lower than that re-

ported by other authors (Narumya et al., 1986; Liel et al., 1987). The reasons for this difference are uncertain but may stem, at least in part, from not completely identical experimental conditions. Similar discrepancies in binding site number have been reported for U46619 receptors (Liel et al., 1987; Kattelman et al., 1986; Morinelli et al., 1987). The K_d for U46619 that we found was lower than that reported by Kattelman et al. (1986) and Hanasaki et al. (1988), but was similar to that observed by Liel et al. (1987).

Picotamide was found to inhibit the binding of the two radiolabelled thomboxane A_2 analogues, [^{125}I]PTA-OH and [^{3}H]U46619. The inhibitory constant for picotamide gives an index of the potency of the compound to inhibit binding. Gresele et al. (in press) reported that the inhibitory concentration of picotamide on U46619-induced aggregation in vitro was 1.4×10^{-4} (IC_{50}). These authors found a slope of the Schild plot greater than 1, and the IC_{50} was higher than that found in binding inhibition studies. This non-linear effect was attributed to a removal process, which could be saturated at higher concentration, whereas the simple effect on the TxA_2 binding site was evident at lower concentrations. Moreover, the picotamide binding capacity of serum proteins, which are present in the aggregation system but not in the binding studies, may play a role in causing the non-linear effect.

In conclusion, the present investigation provides evidence for a direct inhibitory effect of picotamide at the level of the TxA_2 platelet receptor, which is possibly responsible, at least in part, for the anti-aggregating effect of this drug.

Acknowledgements

We gratefully acknowledge the supply of ONO11120 from Dr. Shuh Narumiya, of the Department of Pharmacology, Kyoto University, Japan.

References

Armstrong, R.A., R.L. Jones and N.H. Wilson, 1983, Ligand binding to thromboxane receptors on human platelets: correlation with biological activity, Br. J. Pharmacol. 79, 953.

Berrettini, M., M. De Cunto, A. Romanelli, I. Volpato and G.G. Nenci, 1983, Studio in vitro sul meccanismo dell' azione antiaggregante piastrinica della picotamide, Boll. Soc. Ital. Biol. Sperim. 59, 309.

Cattaneo, M., M. Chiodini, P.M. Tenconi and P.M. Mannucci, 1988, In vitro effects of Picotamide on human platelet aggregation, the release reaction and thromboxane B2 production, Xth International Congress on Thrombosis, Athens, Abstract Book 169.

Cheng, Y.C. and W.H. Prusoff, 1973, Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction, Biochem. Pharmacol. 22, 3099.

Deckmyn, B.O.H., P. Gresele, E. Van Outte, G.G. Nenci and J. Vermylen, 1985, Synergism of a thromboxane synthetase inhibitor and a receptor antagonist in reducing platelet aggregation, Thromb. Haemostas. 54, 677.

De Cunto, M., M. Berrettini, P. Parise, M. DeRegis, G. Orzalesi and G.G. Nenci, 1983, Studio in vitro dell'effetto di alcuni derivati dell'acido 4-idrossi-isoftalico sull'aggregazione di piastrine umane, Boll. Soc. Ital. Biol. Sperim. 59, 302.

Gresele, P., H. Deckmyn, J. Arnout, G.G. Nenci, J. Vermylen, Characterization of N,N'-bis(3-picolyl)-4-methoxy-isophtalamide (picotamide) as a dual thromboxane synthase inhibitor/thromboxane A2 receptor antagonist in human platelets, Thromb. Haemostas. 61 (3) (in press).

Hanasaki, K. and H. Arita, 1988, Characterization of a new compound, S-145, as a specific TxA_2 receptor antagonist in platelets, Thromb. Res. 50, 365.

Hung, S.C., N.I. Ghali, D.L. Venton and G.C. Le Breton, 1983, Specific binding of the thromboxane A_2 antagonist 13-azaprostanoid acid to human platelet membranes, Biochim. Biophys. Acta 728, 171.

Kattelman, E.J., D.L. Venton and G.C. Le Breton, 1986, Characterization of U46619 binding in unactivated, intact human platelets and determination of binding site affinities of four TxA_2/PGH_2 receptor antagonists, Thromb. Res. 41, 471.

Liel, N., D.E. Mais and P.V. Halushka, 1987, Binding of a thromboxane A_2/Prostaglandin H_2 agonist [^{3}H]U46619 to washed human platelets, Prostaglandins 33, 789.

Mais, D.E., R.M. Burch, D.L. Saussy, P.J. Kochel and P.V. Halushka, 1985a, Binding of TxA_2/PGH_2 receptor antagonist to washed human platelets, J. Pharmacol. Exp. Ther. 235, 729.

Mais, D.E., D.L. Saussy, A. Chaikhouni, P.J. Kochel, D.R. Knapp, N. Hamanaka and P.V. Halushka, 1985b, Pharmacologic characterization of human and canine thromboxane A_2/prostaglandin H_2 receptors in platelets and blood vessels: evidence for different receptors, J. Pharmacol. Exp. Ther. 233, 418.

Modesti, P.A., A. Fortini, R. Abbate and G.F. Gensini, 1985, Age related changes of platelet prostacyclin receptors in humans, European J. Clin. Invest. 15, 204.

Morinelli, T.A., S. Niewiarowski, J.I. Daniel and J.B. Smith, 1987, Receptor mediated effects of PGH_2 analogue (U46619) on human platelets, Am. J. Physiol. 253, H1053.

Narumiya, S., M. Okuma and F. Ushikubi, 1986, Binding of a radioiodinated 13-azapinane thromboxane antagonist to platelets: correlation with antiaggregatory activity in different species, Br. J. Pharmacol. 88, 323.

Neri Serneri, G.G., P.A. Modesti, A. Fortini, R. Abbate, A. Lombardi and G.F. Gensini, 1984, Reduction in prostacyclin platelet receptors in active spontaneous angina, Lancet ii, 838.

Orzalesi, G., R. Selleri and I. Volpato, 1975, Fibrinolysis and inhibition of platelet aggregation by N,N'bis(3-picolyl)-methoxyisophthalamide (G-137), in: Progress in Chemical Fibrinolysis and Thrombosis, Vol. 1, eds. J.F. Davidson, M.M. Samama and P.C. Desnoyes (Raven Press, New York) p. 267.

Scatchard, G., 1949, The attraction of protein for small molecules and ions, Ann. N.Y. Acad. Sci. 51, 660.

Violi, F., A. Ghiselli, L. Iuliano, D. Practico', C. Alessandri and F. Balsano, 1988, Inhibition by Picotamide of thromboxane production in vitro and ex vivo, European J. Clin. Pharmacol. 33, 599.

Weiland, G.A. and P.B. Molinoff, 1981, Quantitative analysis of drug-receptor interactions: I. Determination of kinetic and equilibrium properties, Life Sci. 29, 313.