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SHORT COMMUNICATIONS

Temperature dependence of [^{3}H]PAF binding to washed human platelets

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The thermodynamic analysis of the binding equilibrium of a drug to its receptors allows us to evaluate the forces driving the drug–receptor interaction [1]. The data obtained can contribute to an elucidation of the molecular mechanisms of the interaction and shed further insight on structure–activity relationships [2]. Studies on the temperature dependence of receptor binding constants were carried out on various types of drugs acting at the receptor level (e.g. β-adrenoceptor and dopamine receptor ligands, benzodiazepines, agonists of the opiate receptors) [1, 3]. The thermodynamic parameters observed indicated different entropy- or enthalpy-driven mechanisms.

Platelet activating factor (PAF: 1.*O*-alkyl-2-(*R*)-acetyl-*sn*-glycero-3-phosphorylcholine) is a phospholipid mediator that is synthesized by different cell types and exerts a wide range of pathophysiological effects [4]. PAF activates human platelets by interacting with specific receptors that are labelled by [^{3}H]PAF and blocked by antagonists of different origin and structure [4–6].

With the aim of contributing to an evaluation of the molecular mechanisms underlying PAF-receptor interactions, the entropic and enthalpic factors of PAF binding to its receptors on washed human platelets were ascertained by measuring the thermodynamic parameters of saturation binding assays performed at different temperatures.

Materials and Methods

Platelets. Washed human platelets from the peripheral blood of healthy volunteers were prepared as previously described by Korth *et al.* [5]. The final suspending medium was a Tyrode buffer (pH 7.4) of the following composition (mM) : NaCl 137; KCl 2.68; $NaHCO_3$ 11.9; $MgCl_2$ 1.0; NaH_2PO_4 0.4; glucose 5.5. The reagents were purchased from Merck (Darmstadt, F.R.G.).

Binding assay. Binding of [^{3}H]PAF (1-*C*-[^{3}H]octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, sp. act. 170 Ci/mmol, Amersham, Bucks, U.K.) was measured in a volume of 0.5 mL of Tyrode buffer supplemented with $CaCl_2$ (1.3 mM; Merck) and 0.25% (w/v) BSA (fatty acid-free bovine serum albumin, the Sigma Chemical Co., St Louis, MO, U.S.A.), according to the procedure reported by Korth *et al.* [5]. The platelets (4×10^7 in 0.5 mL) were incubated with increasing concentrations of [^{3}H]PAF (0.05–5 nM) at various temperatures (0°, 15°, 20°, 25°, 30°) and for different periods of time. The length of incubation (2 hr at 0°, 1 hr at 15°, 30 min at 20°, 25° and 30°) was selected in order to achieve equilibrium (data not shown). Bound and free radioligands were separated by rapid vacuum filtration through Whatmann GF/C glass fiber filters (Whatman, Maidstone, U.K.). Filters were then washed twice with 3 mL ice-cold buffer.

Calculations. B_{max} (total number of binding sites) and K_D (dissociation constant) were determined with Scatchard analysis of saturation data by using computer based non-linear curve fitting methods [7]. K_A (affinity constant) was calculated as $1/K_D$. The standard free energy change of the binding reaction was calculated from $\Delta G° = -RT \ln K_A$ at 298°K; the standard enthalpy change of the reaction, $\Delta H°$, from the slope of the Van't Hoff plot $\ln K_A$ vs $1/T$; the standard entropy change of the reaction from $\Delta S° = (\Delta G° - \Delta H°)/T$, where T is the temperature in °K. Statistical significance of binding data was evaluated by means of the Student's t-test.

Results and Discussion

Specific binding of [^{3}H]PAF to washed human platelets achieved saturation at all the temperatures tested when platelets were incubated with the radioligand. Scatchard analysis of the results of a typical experiment performed at 0° is depicted in Fig. 1. Scatchard plots of the saturation binding data were linear at all the temperatures studied (data not shown). Computer analysis [7] of the saturation binding data obtained in the whole range of temperatures failed to show a significantly better fit to a two-site than to a one-site model. These results indicated that only one class of high affinity binding sites for [^{3}H]PAF could be detected on human platelets under our experimental

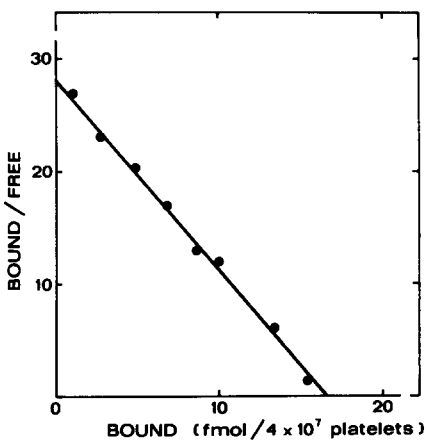

Fig. 1. [^{3}H]PAF binding to washed human platelets: Scatchard plots of typical saturation binding experiments performed at 0°. The calculated K_D and B_{max} were 0.62 nM and 17.8 fmoles per 4×10^7 platelets, respectively.

Table 1. Equilibrium binding parameters for [^{3}H]PAF binding to washed human platelets at various temperatures

Temperature	K_D (nM)	B_{max} (sites/platelet)
0° (273°K)	0.64 ± 0.03	260 ± 18
15° (288°K)	0.41 ± 0.05*	325 ± 42
20° (293°K)	0.35 ± 0.05*	251 ± 25
25° (298°K)	0.27 ± 0.04*	316 ± 37
30° (303°K)	0.26 ± 0.04*	292 ± 30

Data are means ± SE of five separate experiments. The temperatures are expressed both in Celsius (°) and Kelvin (°K) degrees.

* Significantly different from 0°, $P < 0.05$.

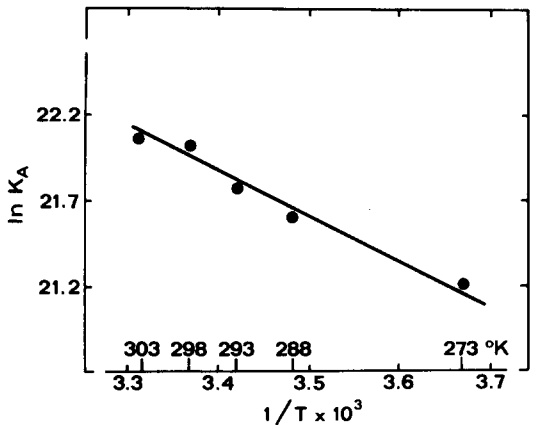

Fig. 2. Van't Hoff plot of [^{3}H]PAF binding to washed human platelets. The values are means of five independent determinations of the affinity constant (K_A) measured as described in Materials and Methods. T is the absolute temperature in °K.

conditions. The calculated K_D and B_{max} values are reported in Table 1. Data obtained at the various temperatures were compared with those collected at 0°. B_{max} values were not significantly changed by increasing the temperature of platelet incubation from 0° to 30°. K_D values decreased when the temperature of the binding experiments was shifted from 0° to 30°; data measured at 15°, 20°, 25° and 30° were significantly different from those calculated at 0°. K_D and B_{max} values we obtained are comparable with those reported by authors who evaluated [^{3}H]PAF binding to washed human platelets at single temperatures within the range we tested [6, 8–10]. While 0° is a temperature able to minimize both the cell incorporation of the radioligand and its metabolism, results obtained by different authors [5, 8–11] have demonstrated that the metabolism of [^{3}H]PAF by washed human platelets was negligible even at the highest temperatures we employed. Therefore, we thought the experimental conditions were suitable for binding studies, even if we did not assay directly the extent of [^{3}H]PAF metabolism by intact platelets. The relationship between changes in K_A, which was calculated from K_D as reported in Materials and Methods, and changes in temperature was expressed by the Van't Hoff plot shown in Fig. 2. The plot was linear in the temperature range evaluated. That allowed us to calculate the $\Delta H°$ of the binding process. The value obtained is reported in Table 2, along with those of the other thermodynamic parameters measured, $\Delta G°$ and $\Delta S°$. The high negative value of $\Delta G°$ is consistent with a high affinity binding of [^{3}H]PAF to its platelet receptors. The positive value of $\Delta H°$ indicates that the binding process is endothermic. The high positive value of $\Delta S°$ shows clearly that [^{3}H]PAF binding to its receptors on human platelets is driven by a large increase in entropy. The calculated values of the thermodynamic parameters are indicative of a reversible chemical process.

Table 2. Equilibrium thermodynamic parameters for [^{3}H]PAF binding to washed human platelets

Temperature range	$\Delta G°$ (kJ/mol)	$\Delta H°$ (kJ/mol)	(J/mol/degree)
273–303°K	−54.18	+21.46	+253.84

In summary, our results indicate that the binding of [^{3}H]PAF to a single class of high affinity sites on human platelets is endothermic and largely entropy-driven. The increase in entropy can be attributed to the disorganization of water molecules bound to both receptor and ligand during the binding process.

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