Inhibition of receptor-mediated platelet activation by nedocromil sodium

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Background: *Platelet activation by platelet activating factor (PAF) seems to be involved in the inflammatory process in asthma and may serve as a possible target for the antiinflammatory drug nedocromil sodium, which is known to inhibit cell activation by different stimuli.*
Methods: *We investigated the effect and the mode of action of nedocromil sodium on platelet activation by PAF. In a set of healthy volunteers* (n = 45) *we investigated seven different parameters of platelet activation by PAF, thrombin, and* Ca^{2+}*-ionophore.*
Results: *Nedocromil sodium inhibited: (1) PAF-induced "shape change" reaction up to 78% (50% inhibitory concentration* $[IC_{50}]$*:* 3×10^{-9} *mol/L), thrombin-mediated "shape change" up to 80%* (IC_{50}*:* 2×10^{-8} *mol/L), but not the* Ca^{2+}*-ionophore–dependent reaction, (2) platelet aggregation by PAF up to 85%* (IC_{50}*:* 2×10^{-9} *mol/L); (3) release of thromboxane* B_2 *up to 82%* (IC_{50}*:* 5×10^{-9} *mol/L); (4) formation of inositol 1,4,5-triphosphate by PAF* (IC_{50}*:* 3×10^{-7} *mol/L), by thrombin* (IC_{50}*:* 1×10^{-7} *mol/L), but not by* Ca^{2+} *ionophore; (5) increase of intracellular free calcium* (IC_{50}*:* 4×10^{-7} *mol/L); (6) formation of diacylglycerol* (IC_{50}*:* 9×10^{-9} *mol/L), and (7) translocation of protein kinase C* (IC_{50}*:* 1×10^{-7} *mol/L).*
Conclusions: *In the concentration range of the* IC_{50} *values found in these experiments, nedocromil sodium reduced PAF binding to platelets by only 10% to 20%, such that this interference cannot explain the observed effects of the compound. Inhibition of receptor-mediated platelet activation at an early stage in the signal transduction pathway, and without effect on* Ca^{2+}*-ionophore–induced platelet activation, suggests an action of nedocromil sodium at the level of the cell membrane. (J Allergy Clin Immunol 1993;91:1217-25.)*

Key words: *Platelet activation, platelet activating factor, thrombin,* Ca^{2+}*-ionophore, nedocromil sodium, receptor specificity*

Recent reports have suggested that platelets play an important role in the inflammatory process in human asthma.[1-7] Stimulation of platelets results in the release of various mediators that may lead to an extension of the inflammation and intensify the immune response.[8-11] Platelet activating factor (PAF) is one of the stimuli potentially involved in platelet activation in asthma. This mediator is of special interest, because it can elicit responses that mimic some aspects of bronchial asthma in human beings and animals (i.e., bronchoconstriction, bronchial hyperresponsiveness, increased vascular permeability, and mucosal edema).[9, 12-16] We have previously shown that inhalation of PAF, which induces a transient bronchoconstriction but no significant increase in airway responsiveness, leads to activation of circulating platelets and to their refractoriness against an additional ex vivo stimulation by PAF. In

Abbreviations used:
PAF: Platelet activating factor
IP_3: Inositol 1,4,5,-triphosphate
$[CA^{2+}]_i$: Concentration of intracellular free calcium
PKC: Protein kinase C
PBS_{sc}: Phosphate-buffered saline supplemented with 0.36% sodium citrate

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addition, a similar state of platelet preactivation, that is increased levels of inositol 1,4,5-triphosphate (IP_3) and intracellular free calcium in circulating platelets, was found in patients with asthma as compared with normal control subjects.[8]

It has been shown that binding of PAF to its membrane receptor activates a G protein (Gp), which leads to activation of phospholipase C, followed by hydrolysis of phosphatidylinositol 4,5-bisphosphate and formation of diacylglycerol as well as IP_3. Inositol triphosphate increases the concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_i$)[17] whereas diacylglycerol activates protein kinase C (PKC).[17, 18] The activation of this second messenger system leads to (1) cell activation,[17] (2) release of a variety of inflammatory mediators, and (3) cell proliferation.[17, 18]

Nedocromil sodium is used therapeutically as an antiinflammatory drug in the treatment of asthma.[19, 20] In vivo studies have demonstrated an inhibitory effect of nedocromil sodium against a range of bronchoconstrictor stimuli.[21, 22] The effects of nedocromil sodium on physiologic cell responses have also been studied in a variety of cell types.[23-37] Thus nedocromil sodium has been shown to reduce the chemotactic effects of PAF on granulocytes.[29-31] Moqbel et al.[32] demonstrated an inhibition of eosinophil activation by formyl-methionyl-leucyl-phenylalanine or calcium ionophore A23187. Additionally, nedocromil sodium reduces the release of leukotriene C_4 from eosinophils, stimulated by calcium ionophore A23187[33, 34]; and the release of leukotriene B_4 and 5-hydroxy-eicosatetraenoic acid in lung macrophages of aspirin-sensitive patients with asthma.[34-36]

So far, relatively few studies have investigated the effects of nedocromil sodium in platelets. Thorel et al.[23] reported that nedocromil sodium affects the IgE-dependent antiparasite functions of rat macrophages and platelets; additionally, an inhibitory effect of nedocromil sodium on IgE-mediated activation of human mononuclear phagocytes and platelets was shown.[24] Marquette et al.[25] showed a preventive effect of nedocromil sodium on the abnormal response to aspirin of platelets from aspirin-sensitive patients with asthma.

In the present study we determined the effect of nedocromil sodium on PAF-induced platelet activation by assessing the shape change reaction, platelet aggregation, and thromboxane A_2 generation. Shape change and aggregation are final responses of platelets that follow activation by different stimuli, and both reactions can be induced by PAF.[8, 37] Because nedocromil sodium was reported to block the arachidonic acid pathway,[35] we also assessed the PAF-induced release of thromboxane A_2. To further investigate the mechanism of interaction of nedocromil sodium with platelet activation, we studied its effect on the intracellular signal transduction pathway by measuring the formation of IP_3, changes in the $[Ca^{2+}]_i$, the formation of diacylglycerol, and the translocation of PKC. We attempted to further investigate the mechanism of inhibition of platelet activation by nedocromil sodium by using the nonspecific agent, Ca^{2+} ionophore A23187 and a second receptor-mediated agonist, thrombin. For these experiments an effector response (aggregation) and a second messenger response (IP_3 formation) were assessed. Finally, we attempted to exclude an effect of nedocromil sodium on the specific binding of PAF to its membrane receptor, as a possible mechanism of action of the compound.

METHODS

Chemical agents

Nedocromil sodium was supplied by Fisons (Pharmaceutical Division, Loughborough, Leicestershire, England). For each experiment, a fresh stock solution (1×10^{-3} mol/L) of nedocromil sodium was prepared in various assay buffers. PAF and lyso-PAF were purchased from Nova Biochem (Luzern, Switzerland) and prepared as described by Block et al.[8] For each experiment fresh stock solutions (1×10^{-3} mol/L) of both drugs were prepared daily. Tritium-labeled PAF was purchased from Amersham International Ltd. (Little Chalfont, Bucks, England); thrombin and Ca^{2+}-ionophore-A23187 were purchased from Sigma (Sigma-Chemie, Buchs, Switzerland).

Isolation of platelets

Venous blood (30 ml; anticoagulant: 0.36% sodium citrate) was collected from healthy volunteers. The platelets were isolated by centrifugation at 350 *g* for 20 minutes at room temperature. The supernatant (platelet rich plasma) was transferred to another tube and centrifuged a second time at 750 *g* (5 minutes at room temperature). The pelleted platelets were resuspended in 10 ml of phosphate-buffered saline supplemented with 0.36% sodium citrate (PBS_{sc}) followed by a third centrifugation. Then the pellet was resuspended in 1 ml of one of the appropriate assay buffers (see below). The platelets were counted with the aid of a thrombocytometer, and their number was adjusted to the required density.

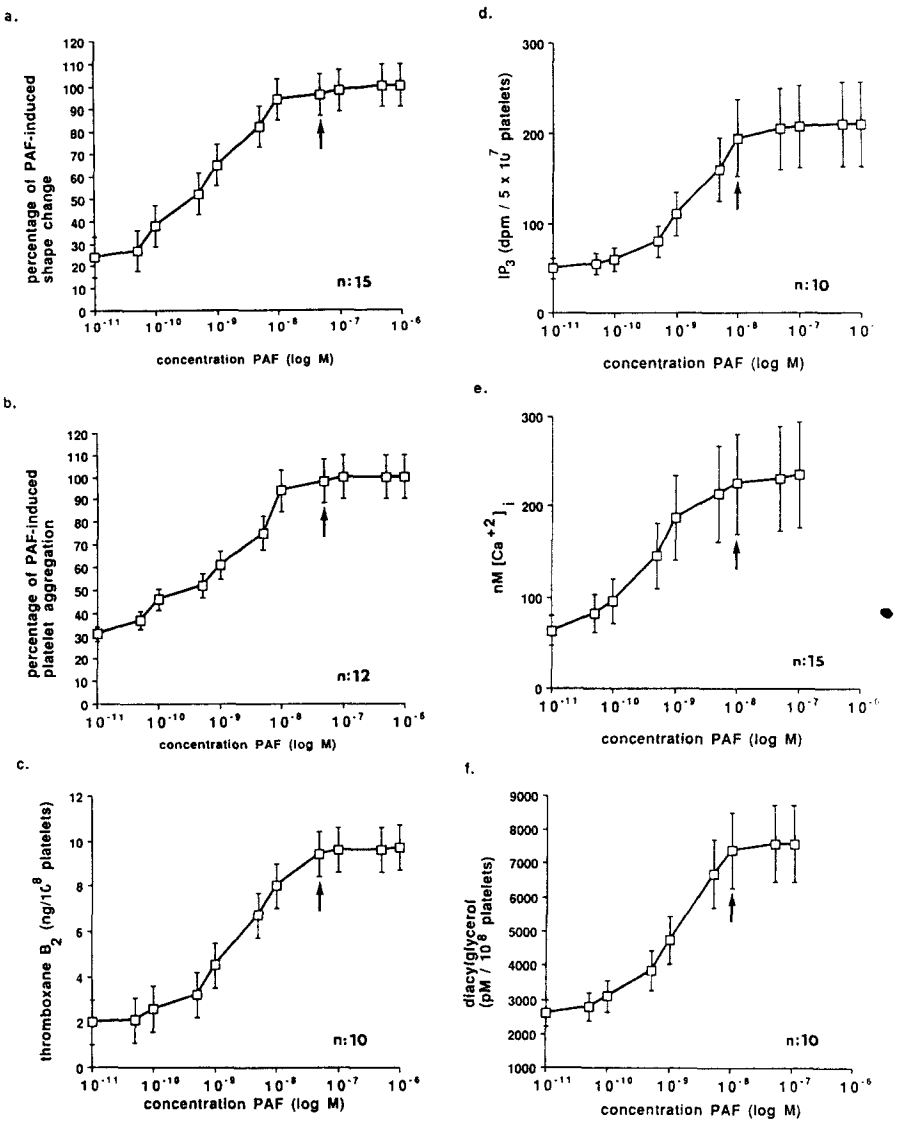

FIG. 1. Functional responses of platelets to various concentrations of PAF. **a,** Shape change reaction; **b,** platelet aggregation; **c,** generation of thromboxane B_2; **d,** formation of IP_3; **e,** increase in intracellular free Ca^{2+}; and **f,** formation of diacylglycerol in human platelets. Each data point represents the mean ± SD. *Arrows* indicate the concentration of PAF used for the stimulation experiments in the presence of nedocromil sodium.

Platelet stimulation by PAF

For all parameters of platelet activation a dose-response curve of different concentrations of PAF (10^{-11} mol/L to 10^{-6} mol/L) was established, and an optimal concentration of the stimulus for the respective parameter of activation was selected (Fig. 1).

Preincubation of platelets with nedocromil sodium

Before challenge, platelets were incubated for 30 minutes (37° C) with various concentrations of nedocromil sodium (10^{-6} to 10^{-12} mol/L) and dissolved in the appropriate assay buffer or the solvent buffer alone (positive control). Platelets were then challenged with PAF (1×10^{-8} mol/L or 5×10^{-8} mol/L) as described in detail for the different measurements. Platelets challenged with lyso-PAF (1×10^{-8} mol/L) and nonstimulated platelets were used as negative controls. All experiments were done in triplicate.

Platelet "shape change"

Platelets from 15 healthy blood donors were used to study the "shape change" reaction induced by PAF (final concentration: 5×10^{-8} mol/L), thrombin

(1×10^{-8} mol/L), or Ca^{2+} ionophore (1×10^{-8} mol/L). In brief, platelets (1×10^8 ml) were resuspended in Tyrode's buffer (10 mmol/L Hepes, 145 mmol/L NaCl, 5 mmol/L KCl, 5 mmol/L $MgSO_4$, 500 μmol/L NaH_2PO_4, 6 mmol/L glucose at pH 7.4), and shape change was monitored by absorbance with an aggregometer connected to an analog chart recorder. The percentage of shape change and its inhibition by nedocromil sodium was calculated as follows[38]:

$$\text{Activation}\% = \frac{A_1 - A_{non}}{A_{max} - A_{non}} \times 100$$

where A_1 is the obtained absorbance value obtained after stimulation with PAF (1×10^{-8} mol/L) in the presence of nedocromil sodium, A_{non} represents the background value (obtained absorbance) in unstimulated platelets, and A_{max} is the obtained absorbance value obtained after stimulation with PAF (1×10^{-8} mol/L) alone.

Platelet aggregation

Platelet aggregation was studied in 12 subjects. Platelet rich plasma was prepared as previously described.[38] Platelets (1×10^9/ml) were challenged with 5×10^{-8} mol/L PAF, and the aggregatory response of the platelets was monitored by absorbance with an aggregometer connected to an analog chart recorder. The percentage of aggregation and its inhibition by nedocromil sodium was calculated in a manner similar to that for "shape change."[38]

Release of thromboxane A_2

The release of thromboxane A_2 from platelets after PAF stimulation was evaluated in 10 subjects by measuring thromboxane B_2, a stable metabolite of thromboxane A_2. Platelets (1×10^8) were stimulated by PAF (5×10^{-8} mol/L), and centrifuged after 10 minutes (1000 *g* for 10 seconds). The amount of released thromboxane B_2 was quantified in the supernatant by a specific radioimmunoassay system (Amersham) according to the instructions of the distributor.

IP_3

Platelets from 10 healthy blood donors were used to study the formation of IP_3. Platelets (1×10^8/ml) were preincubated for 1 hour with [^{3}H]-*myo*-inositol and nedocromil sodium (30 minutes) at 37° C, followed by challenge with PAF (1×10^{-8} mol/L), thrombin (1×10^{-8} mol/L), or Ca^{2+}-ionophore (1×10^{-8} mol/L). In a prestudy the formation of mono-, di-, and triphosphates was determined by stepwise elution from a Dowex column (Bio-Rad Laboratories, Chemical Div., Richmond, Calif.), which was followed by liquid scintillation counting.[38] Later on, IP_3 was determined by a specific radioimmunoassay system (Amersham).

Intracellular free calcium concentration

The $[Ca^{2+}]_i$ was assessed in platelets from 15 healthy subjects. Platelets (1×10^8/ml) were incubated with one of two different calcium labels (fura 2-acetoxymethyl ester or Quin-2 [Sigma-Chemie]) for 30 minutes (37° C), preincubated with nedocromil sodium, and then challenged with PAF (1×10^{-8} mol/L). The changes of the intracellular calcium concentrations were determined as described by Erne et al.[39] and the ratio of the emitted light signal was assessed with a fluorescence photospectrometer (340 nm/380 nm).

Diacylglycerol

The formation of diacylglycerol was determined in platelets obtained from 10 healthy volunteers with a competitive radioimmunoassay (Amersham). Platelets (1×10^9/ml) were challenged with PAF (1×10^{-8} mol/L) for 1 minute; then the reaction was stopped by addition of 1 ml 10% trichloroacetic acid, and diacylglycerol was measured as described in the distributor's manual (Amersham).

PKC

The translocation of PKC was assessed in platelets (1×10^9/ml) from three healthy volunteers challenged with PAF (1×10^{-8} mol/L). In brief, platelets were pelletted by centrifugation and resuspended in an incubation buffer (20 mmol/L TRIS base, 1 mmol/L $CaCl_2 \times 2\ H_2O$, 0.1 mmol/L magnesium acetate) and sonicated. Membraneous and cytosolic fractions were separated by ultracentrifugation (34.000 *g*, 1 hour, at 4° C) and supplemented with polymethylsulfonyl fluoride (final concentration = 1 mmol/L), Nonidet P40 (1%) (Sigma-Chemie). Samples were incubated on ice (30 minutes) followed by purification over a DE-52 column (Bio-Rad Laboratories, Chemical Div.) and eluted in 1.2 ml of 80 mmol/L NaCl + 1 mmol/L polymethylsulfoxyl fluoride. The eluate (50 μl) was mixed with 75 μl incubation buffer (supplemented with 0.5% bovine serum albumin, 100 μmol/L dithiothreitol, 15 μg phosphatidylserine, 5 μg diolein, 50 μCi phosphorous-32–labeled adenosine triphosphate. The phosphorylation of histone was determined after trichloroacetic acid precipitation on a nitrocellulose filter followed by liquid scintillation counting.[38]

Inhibition of ^{3}H-PAF binding to platelets by nedocromil sodium

Platelets (1×10^8) from 10 healthy subjects were isolated and preincubated with various concentrations of nedocromil sodium (1×10^{-10} to 1×10^{-5} mol/L in PBS_{sc}) for 30 minutes. After washing with PBS_{sc}, platelets were resuspended in 1 ml of PBS_{sc}, and ^{3}H-PAF (1×10^{-8} mol/L) was added for 60 minutes (37° C). Platelets were washed once with PBS_{sc} and resuspended in 10 ml of liquid scintillation buffer. The amount of bound ^{3}H-PAF was determined as counts per minute.[40]

To determine the specificity of the binding of ^{3}H-PAF to its receptor, we additionally tested the replacement of tritium-labeled PAF (1×10^{-8} mol/L) by various concentrations of unlabeled PAF according to the method of Kloprogge and Akkerman.[40]

Statistical analysis

Statistical analysis was performed with a personal computer program (Stateview, Apple MacIntosh, Apple Computer Inc., Cupertino, Calif.), according to the instructions by Sachs.[41] Analysis of variance was performed by the Mann-Whitney U test and Student's *t* test. Values were given as means ± SD.

RESULTS

Stimulation of platelets with the concentrations of PAF used in these experiments has previously been demonstrated to provide reproducible, maximal responses (Fig. 1). All experiments were done in triplicate. When comparing different methods of isolation of platelets (e.g., in the presence of ethylenediaminetetraacetic acid or by gel filtration[39]), we observed no significant differences related to the method used in this series of experiments (data not shown).

The shape change reaction in response to PAF was inhibited by nedocromil sodium with a maximal effect of 78% inhibition at a concentration of $5 \pm 6 \times 10^{-7}$ mol/L. In comparison, shape change induced by thrombin was inhibited by 80% ($1 \pm 5 \times 10^{-6}$ mol/L), whereas Ca^{2+}-ionophore–induced "shape change" was not significantly inhibited by nedocromil sodium at any concentration. No "shape change" reaction was induced when lyso-PAF was used as a stimulus (Fig. 2, *a*). The 50% inhibitory concentration (IC_{50}) values are given in Table I.

Nedocromil sodium inhibited PAF-induced platelet aggregation by up to 85% at a concentration of $1 \pm 0.3 \times 10^{-7}$ mol/L and PAF-mediated generation of thromboxane B_2 up to 82% at a concentration of $5 \pm 0.4 \times 10^{-6}$ mol/L. No platelet aggregation or release of thromboxane B_2 was induced by lyso-PAF (Fig. 2, *b* and *c*).

Nedocromil sodium completely inhibited the formation of IP_3 in response to PAF at a concentration of $6 \pm 3 \times 10^{-7}$. Thrombin-induced IP_3 formation was completely blocked at a concentration of $5 \pm 0.3 \times 10^{-6}$ mol/L. However, the drug did not significantly influence IP_3 formation after Ca^{2+}-ionophore A23187 stimulation (Fig. 3, *a*); IC_{50} values are shown in Table I. In addition, the inhibition of IP_3 formation was not accompanied by an increase of mono- or diphosphates.

The changes in $[Ca^{2+}]_i$ after PAF stimulation were completely inhibited by nedocromil sodium at $9 \pm 3 \times 10^{-7}$ mol/L. As with IP_3 formation, the effect of nedocromil sodium occurred within a very small concentration range. No differences in $[Ca^{2+}]_i$ levels were observed, when nonstimulated platelets and lyso-PAF–challenged platelets were compared (Fig. 3, *b*).

The formation of diacylglycerol was completely inhibited by nedocromil sodium at concentrations greater than $4 \pm 0.3 \times 10^{-6}$ mol/L (Fig. 3, *c*).

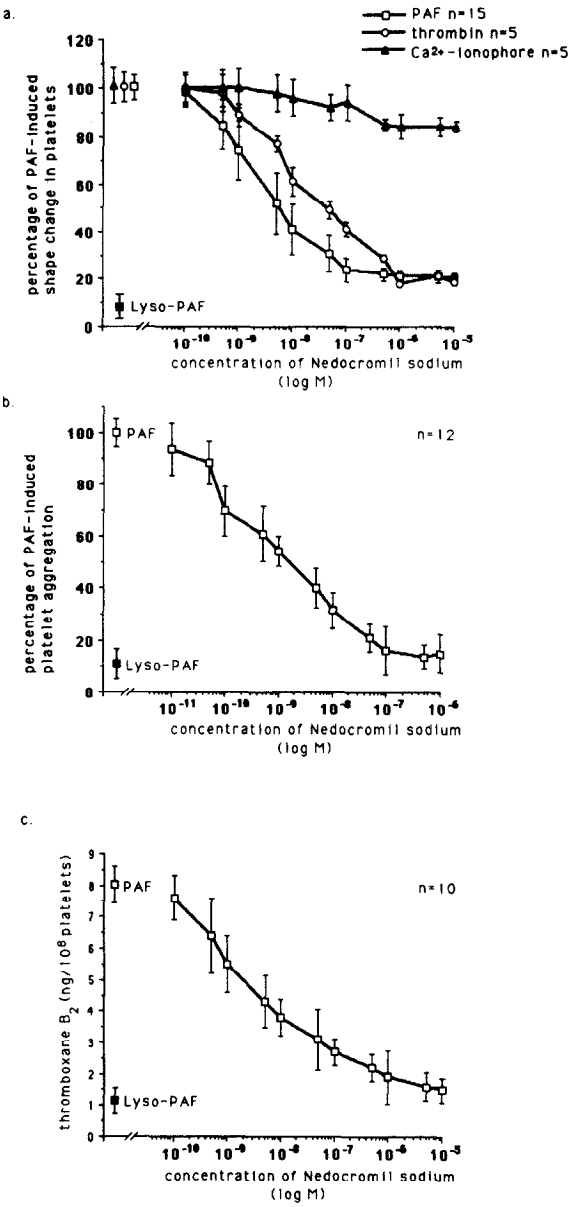

FIG. 2. Functional responses of platelets in the presence of nedocromil sodium. **a,** Inhibition of shape change reaction of human platelets in response to PAF (5×10^{-8} mol/L), thrombin (1×10^{-8} mol/L), and Ca^{2+}-ionophore A-23187 (1×10^{-8} mol/L) after preincubation with different concentrations of nedocromil sodium. **b,** Inhibition of PAF-induced (5×10^{-8} mol/L) platelet aggregation by nedocromil sodium. Lyso-PAF (5×10^{-8} mol/L) was used as negative control. **c,** Dose-dependent inhibition of thromboxane B_2 generation by preincubation with nedocromil sodium in PAF-stimulated (5×10^{-8} mol/L) platelets. Lyso-PAF (5×10^{-8} mol/L) was used as negative control. Each data point represents the mean ± SD.

TABLE I. The 50% inhibitory concentrations (IC_{50}), the maximal inhibitory concentrations (IC_{max}), and the maximal inhibitory effect of nedocromil sodium in human platelets challenged with PAF, thrombin, and CA^{2+}-ionophore A23187

Parameters of platelet activation	Stimulus	n	50% inhibitory concentration (IC_{50})	Maximal inhibitory concentration (IC_{max})	Maximal achievable inhibition (%)
Shape change	PAF	15	$3 \pm 5.0 \times 10^{-9}$ mol/L	$5 \pm 6.0 \times 10^{-7}$ mol/L	78
	Thrombin	5	$2 \pm 2.0 \times 10^{-8}$ mol/L	$1 \pm 5.0 \times 10^{-6}$ mol/L	80
	Ca^{2+}-ionophore	5	No inhibition	No inhibition	–
Aggregation	PAF	12	$2 \pm 0.5 \times 10^{-9}$ mol/L	$1 \pm 3.0 \times 10^{-7}$ mol/L	85
Thromboxane B_2	PAF	10	$5 \pm 0.4 \times 10^{-9}$ mol/L	$5 \pm 0.4 \times 10^{-6}$ mol/L	82
IP_3	PAF	10	$3 \pm 2.0 \times 10^{-7}$ mol/L	$6 \pm 3.0 \times 10^{-7}$ mol/L	100
	Thrombin	5	$1 \pm 0.8 \times 10^{-7}$ mol/L	$1 \pm 3.0 \times 10^{-6}$ mol/L	100
	Ca^{2+}-ionophore	5	No inhibition	No inhibition	–
$[Ca^{2+}]_i$	PAF	15	$4 \pm 0.5 \times 10^{-7}$ mol/L	$9 \pm 3.0 \times 10^{-7}$ mol/L	100
Diacylglycerol	PAF	10	$9 \pm 3.0 \times 10^{-9}$ mol/L	$4 \pm 3.0 \times 10^{-6}$ mol/L	100
PKC	PAF	3	$1 \pm 6.0 \times 10^{-7}$ mol/L	$1 \pm 8.0 \times 10^{-6}$ mol/L	–

Lyso-PAF–challenged platelets did not reveal any significant stimulatory response.

PKC activation, which is dependent on a translocation of PKC from the cytosol to the membrane, was determined in only three experiments. The results indicate a blocking effect of nedocromil sodium on PAF-mediated activation of PKC (Fig. 3, *d*). No translocation was observed when platelets were challenged with lyso-PAF.

As shown in Fig. 4, nedocromil sodium reduced the binding of ^{3}H-PAF to platelets up to 35%, at a concentration of 1×10^{-5} mol/L. In the concentration range of the IC_{50} values used in the experiments described above (1×10^{-7} to 1×10^{-9} mol/L), nedocromil sodium reduced PAF binding to platelets by only 10% to 20% (Fig. 4). This degree of interference with PAF binding to its receptor would still lead to maximal platelet activation, as can be derived from the concentration response curves in Fig. 1. Interference with PAF binding therefore is not responsible for the blocking effect of nedocromil sodium on platelet activation by PAF.

DISCUSSION

We have demonstrated an effect of nedocromil sodium on PAF-induced platelet activation resulting in inhibition of shape change, aggregation, thromboxane A_2 generation, as well as inhibition of second messenger signals (IP_3 formation $[Ca^{2+}]_i$, diacylglycerol synthesis, and translocation of PKC). The drug also interfered with platelet activation by thrombin, a specific receptor-mediated stimulus, whereas it had no effect on Ca^{2+}-ionophore A23187–induced shape change response and IP_3 formation. In addition, we have shown that nedocromil sodium, at the concentrations that were effective in these experiments, only slightly interferes with the binding of PAF to platelets, in the range of 10% binding reduction. Therefore this finding cannot explain the effects of the drug on the investigated parameters for signal transduction. The results of these experiments indicate that nedocromil sodium may act at the cell membrane to inhibit receptor-mediated platelet activation by preventing subsequent intracellular events.

Nedocromil sodium has been shown to interfere with different types of cell activation, such as neutrophil and eosinophil chemotaxis[28, 30, 31] and mediator secretion in response to PAF, formyl-methionyl-leucyl-phenylalanine, or Ca^{2+} ionophore A23187.[32-36] These effects can be compared with the functional responses of platelets such as aggregation, shape change, and thromboxane A_2 secretion. In our experiments, IC_{50} values and maximal inhibitory concentrations were 10 to 100 times lower than those in the above mentioned studies. This may be due to several factors: differences in concentrations of the activating stimulus or in the sensitivities of the physiologic response systems under the given experimental conditions may be involved. In our system, different concentrations of PAF were necessary to induce reproducible maximal responses, depending on the concentration of platelets in the assay. Complex biologic responses, such as chemotaxis, may depend on specific experimental conditions, such as serum content of the medium; the latter may influence the activity of nedocromil sodium.

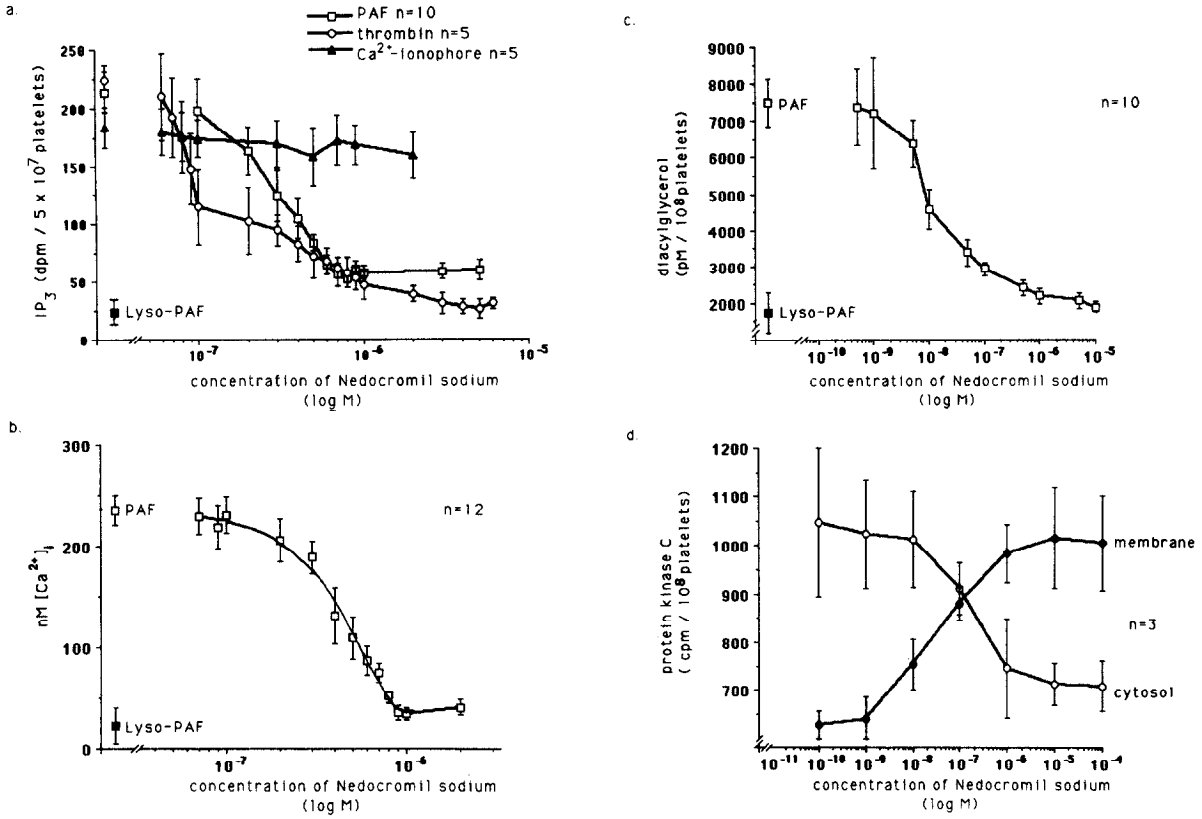

FIG. 3. Second messenger signals in platelets. **a,** Effect of nedocromil sodium on IP_3 in platelets in response to PAF (1×10^{-8} mol/L), thrombin (1×10^{-8} mol/L), and Ca^{2+}-ionophore A-23187 (1×10^{-8} mol/L). Lyso-PAF (1×10^{-8} mol/L) was used as negative control. **b,** The effect of nedocromil sodium on PAF-induced (1×10^{-8} mol/L) increase in intracellular free Ca^{2+}. Lyso-PAF (1×10^{-8} mol/L) was used as negative control. **c,** Effect of nedocromil sodium on PAF-induced (1×10^{-8} mol/L) diacylglycerol formation in human platelets. Lyso-PAF (1×10^{-8} mol/L) was used as negative control. **d,** Effect of nedocromil sodium on PAF-induced (1×10^{-8} mol/L) translocation of PKC in human platelets. Lyso-PAF (1×10^{-8} mol/L) was used as negative control. Each data point represents the mean ± SD.

In our experiments we repeatedly found that the addition of serum enhanced the blocking effect of nedocromil sodium (results not shown).

An important difference may also be due to the "all or nothing" reaction of platelets in response to different stimuli and the short response times of several minutes, as opposed to cells with a complete genome and enzymatic apparatus, which can vary their complex reactions, taking more time to occur. It is important therefore to emphasize that our results correspond quite well with other experiments in platelets, such as the suppression of IgE-dependent platelet activation with an IC_{50} of 2×10^{-9} mol/L for nedocromil sodium.[23]

The maximal inhibitory concentrations of nedocromil sodium were in a similar range for the functional responses as for the second messenger signals. However, the maximal inhibitory effect was in the range of 80% for functional changes,

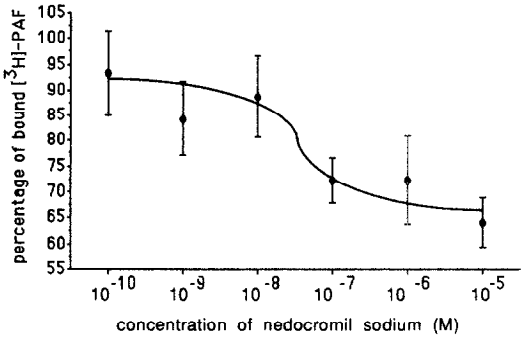

FIG. 4. Inhibition of ^{3}H-PAF binding to isolated human platelets (1×10^8) in the presence of various concentrations of nedocromil sodium.

whereas the second messenger signals were completely suppressed. This may be due to different concentrations of PAF, in relation to various platelet concentrations, in the different assay systems to achieve reproducible maximal responses.

Furthermore, there may be additional second messenger pathways involved, which were not investigated in our experiments. These, however, would seem to be of minor physiologic importance. Only a small number of studies have investigated the effects of nedocromil sodium on second messengers; but in contrast to our findings, no inhibitory effect on receptor-mediated increase of intracellular $[Ca^{2+}]_i$ was observed.[32, 34] Bruijnzeel et al.[34] explained their findings with a possible inhibition of PKC by nedocromil sodium, as postulated by others.[42] Our demonstration of an inhibitory effect of nedocromil sodium on PKC activation by PAF is in accordance with these findings. However, our data suggest an inhibition at an earlier stage of the intracellular signal transduction pathway, since the formation of IP_3, diacylglycerol, and $[Ca^{2+}]_i$ by PAF, as well as IP_3 formation, in response to thrombin were inhibited.

Because nedocromil sodium blocked thrombin- and PAF-mediated but not Ca^{2+}-ionophore–induced platelet activation, we hypothesize an interaction of the compound at the level of the cell membrane, thereby blocking the intracellular signal transmission after activation of different receptors. An extracellular action of nedocromil sodium, a highly polarized molecular unlikely to enter the cell, is also compatible with the observation that the effect of the compound can be reduced by repeatedly washing the cells.[40] We observed a similar effect in all test systems when pretreated platelets were washed more than twice with buffer (data not shown).

The possibility of an interference of nedocromil sodium with PAF binding to its membrane receptor was excluded in a separate set of experiments. At concentrations that were effective in the activation studies, the drug showed only minor interference with PAF binding to platelets. A 10% to 20% decrease in PAF binding would result in a minor shift of the concentration-activation relationship (Fig. 1) but would not explain the inhibition of up to 80% of the platelet response to PAF.

Histologic[43, 44] and bronchoalveolar lavage studies[45] have repeatedly indicated the involvement of platelet activation in bronchial asthma. Platelets have a well-recognized potential to release proinflammatory mediators[8-11, 46] and may therefore enhance the inflammatory process in asthma. Inhaled nedocromil sodium has been demonstrated to interfere with functional responses of circulating platelets.[25] The inhibition of platelet activation demonstrated in this study may therefore contribute to the antiinflammatory effects of nedocromil sodium in asthma.

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