Volume 312, number 2,3, 200–202 FEBS 11730 November 1992

The NK_1 receptor is involved in the neurokinin-induced shape change of rabbit platelets

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Received 7 September 1992; revised version received 23 September 1992

Substance P and selective neurokinin receptor agonists have been tested for their ability to induce shape change in rabbit platelets. Substance P and the NK_1 receptor agonist Ac [Arg^6,Sar^9,$Met(O_2)^{11}$]-substance P (6–11) induced shape change (EC_{50} = 3 and 6 nM, respectively), whereas the selective NK_2 agonist [Nle^{10}]-Neurokinin A (4–10) and the selective NK_3 agonist [$MePhe^7$]-Neurokinin B did not show any effect. Moreover, the specific NK_1 receptor antagonist CP-96,345 selectively and dose-dependently counteracted the effect of substance P or of the NK_1 receptor agonist (IC_{50} = 2 and 0.8 nM, respectively), whereas the selective NK_2 receptor antagonist, SR 48968, had no effect. Unlike for serotonin or low doses of ADP, epinephrine did not allow substance P or the NK_1 receptor agonist to become a proaggregating substance. These data therefore show that the NK_1 receptor is solely involved in the neurokinin-induced shape change of rabbit platelets.

Substance P; Neurokinin; Neurokinin receptor agonist; Rabbit platelet; Shape change in rabbit platelet

1. INTRODUCTION

Neurokinins are endogenous peptides present in the central and peripheral nervous system [1]. Their functions have not yet been clearly elucidated, but they seem to be involved in a bi-directional communication between the immune and the nervous system [1]. Three principal types of natural occurring neurokinins have been described: substance P [2,3], neurokinin A and B [4]. The three neurokinins act, with imperfect specificity, on three different types of receptors, respectively named NK_1, NK_2 and NK_3, which have been cloned, characterized biochemically as well as pharmacologically [5–10].

The biological effects of neurokinins have been essentially described on smooth muscle system (respiratory, gastrointestinal, cardiovascular and urinary) and nervous system (gland secretion, transmission of painful stimuli) [11] but additional properties suggest that neurokinins are also involved in immunological and inflammatory process including proliferation of lymphocytes T [1], leukocytes migration, neutrophils degranulation [12], phagocytosis [13] and endothelial gap formation [12].

Moreover, neurokinins produce diverse effects on platelets, including shape change [14], potentiation of cytotoxicity against *Schistosoma mansini* [15] in vitro and intra vessel platelet aggregate formation in vivo [12]. Gudat et al. [14] indicated that substance P-induced shape change of rabbit platelets was the result of a specific interaction of the agonist with a receptor but did not further characterize the exact type of receptor mediating the interaction of substance P with platelets.

The aim of this study was thus to demonstrate what type of neurokinin receptor was mainly involved in the neurokinin-induced platelet shape change using specific neurokinin receptor agonists and antagonists.

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2. MATERIALS AND METHODS

2.1. *Drugs and materials*

Ac[Arg^6,Sar^9,$Met(O_2)^{11}$]-substance P (6–11) (NK_1 ago), [Nle^{10}]-Neurokinin A (4–10) (NK_2 ago) and [$MePhe^7$]-Neurokinin B (NK_3 ago) were purchased from Novabiochem (Läufelfingen, Switzerland). Substance P, serotonin (5-HT) and epinephrine were from Sigma (l'Isle d'Abeau, France). Adenosine 5'-diphosphate (ADP) was provided by Boehringer Mannheim (Meylan, France). CP-96.345, a selective NK_1-specific antagonist and SR 48968, a selective NK_2-specific antagonist were synthesized in the laboratories of Sanofi Recherche (Montpellier, France) as previously described [16].

2.2. *Preparation of platelet-rich plasma*

Blood was withdrawn by venipuncture from male New-Zealand rabbits (4–5 kg, Lago, France). The sample (72 ml) was immediately mixed with 8 ml of a 3.8% tri-sodium citrate solution and platelet-rich plasma (PRP) was obtained by centrifugation (10 min, 300 × *g*, 15°C). The pellet was then further centrifuged (2,000 × *g*, 10 min) to obtain platelet poor plasma (PPP). Platelet concentration of the PRP was adjusted to 700,000 cells/μl by adding PPP. PRP was then incubated at 37°C for 2 h.

2.3. *Measurement of platelet shape change and aggregation*

Shape change was measured by the increase of the light absorption in an aggregometer, according to Colman et al. [17]. PRP (400 μl) was placed for 2 min in the cuvette of a dual-channel aggregometer (Chronolog Corp., Havertown, PA) under constant stirring (900 rpm) at 37°C. Then, 4 μl of a 500 mM EDTA solution (pH 7.4) were added

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and, 10 s later, shape change was induced by the various agonists at the indicated concentrations. Shape change was expressed as the percent of maximal light absorption induced by 10 μM ADP, in the same experimental conditions.

Aggregation was measured and expressed according to the turbidimetric method of Born and Cross [18]. PRP (400 μl) was placed in the aggregometer at 37°C, under constant stirring as described above. The various agonists were incubated with the PRP for 1 min and the aggregation was induced by the addition of epinephrine (10 μM). The aggregation was expressed as the percent of the light transmission, where the OD of the PRP was 0% and the OD of the PPP was 100% of aggregation.

3. RESULTS AND DISCUSSION

Substance P and the selective NK_1 receptor agonist Ac[Arg^6,Sar^9,$Met(O_2)^{11}$]-substance P (6–11) (NK_1 ago) induced shape change of rabbit platelets (Fig. 1). This effect was significant from the dose of 1–3 nM and reached a maximum at the concentration of 1 μM. The EC_{50} (concentrations which exhibited 50% of the maximal effect) of substance P and the NK_1 ago were respectively of 3 and 6 nM. After the addition of 10 μM of substance P or the NK_1 agonist, the observed shape change represented ≈30% of what could be obtained after stimulation with ADP or 5-HT at the same dose.

In contrast, neither [Nle^{10}]-Neurokinin A (4–10) (NK_2 ago) nor [$MePhe^7$]-Neurokinin B (NK_3 ago) did show any effect on the shape change of rabbit platelets therefore showing that this effect was specific only mediated by the NK_1 receptor.

In order to ascertain such an observation, two neurokinin receptor antagonists were tested for their ability to affect NK_1 agonist- or substance P-induced shape change. CP-96,345 is a non-peptide antagonist which has been shown to specifically inhibit the binding of

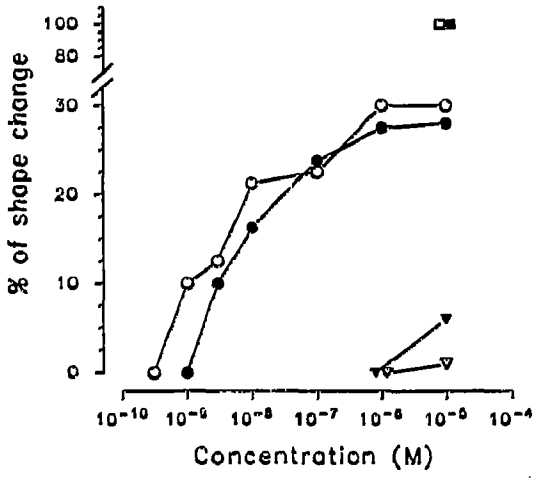

Fig. 1. Shape change of the rabbit platelets, induced by neurokinins, serotonin and ADP. Rabbit platelet shape change was measured after the addition of increasing concentrations of substance P (○), NK_1 ago (●), NK_2 ago (▽), NK_3 ago (▼), 5-HT (□) or ADP (■). Results are expressed as mean % of shape change obtained with 10 μM ADP (n = 4).

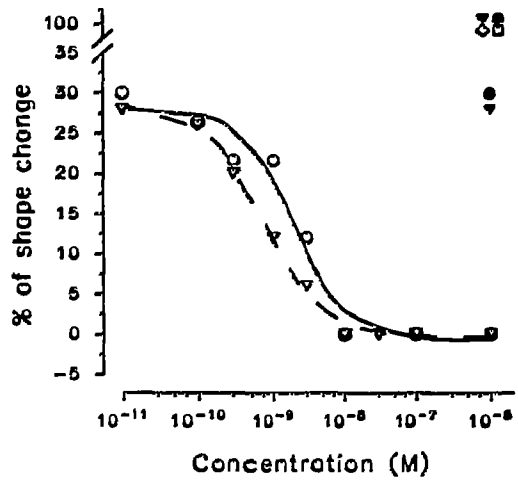

Fig. 2. Effects of neurokinin antagonists on rabbit platelet shape change. Rabbit PRP was incubated for 2 min with the specific NK_1 receptor antagonist CP-96,345 (empty symbols) or the specific NK_2 receptor antagonist SR 48968 (full symbols) at the indicated concentrations. Shape change was then measured after the addition of 1 μM substance P (circles), 1 μM NK_1 ago (triangles), 10 μM 5-HT (squares) or 10 μM ADP (diamonds). Results shown are means (n = 4).

[^{3}H]substance P to the NK_1 receptor [13]. It also inhibited the effects of substance P at nanomolar concentrations in numerous animals model [19]. As shown in Fig. 2, CP-96,345 counteracted in a dose-dependent manner the effect of 1 μM substance P or the NK_1 agonist with IC_{50} values (concentration which inhibited 50% of the effect of the agonist) of 2 and 0.8 nM, respectively. These effects were highly specific since CP-96,345 failed to inhibit the shape change induced either by 5-HT or ADP (10 μM).

SR 48968 is a non-peptide compound which has been recently described to potently and selectively inhibit the binding of [^{125}I]NKA to the NK_2 receptor on various cells and tissues without affecting the binding of specific agonists of the NK_1 and NK_3 receptors [20]. These effects and their selectivity were recently confirmed in a variety of isolated smooth muscle cell preparations [21]. In our experimental conditions, SR 48968 did not inhibit substance P- or the NK_1 agonist-induced shape change therefore confirming that the substance P-induced shape change of rabbit platelets was mediated by the NK_1 receptor. It is noteworthy that neither CP 96,345 nor SR 48,968 did induce shape change per se.

As already described in numerous studies [22], shape change of platelets often precludes to platelet aggregation which seems to occur as a consecutive event. Indeed, several works from our laboratory and others [23] showed that in rabbit platelets, 5-HT or non-aggregating amounts of ADP induced platelet shape change without triggering platelet aggregation which occurred in synergy with epinephrine. For these reasons, substance P or the selective NK_1 agonists were incubated in the presence of epinephrine and the aggregating re-

sponse of platelets was measured. As already shown [24], epinephrine (10 μM) did not induce platelet aggregation by itself but potentiated the aggregating effect of 5-HT or ADP. In contrast, when platelets were first stimulated with substance P or the NK_1 ago, epinephrine did not allow them to become proaggregating agents (data not shown).

In conclusion, our study demonstrates that neurokinin-induced shape change of rabbit platelets is only mediated by the NK_1 receptor. This shape change is not linked to platelet aggregation since epinephrine did not synergize with substance P or the NK_1 agonist to trigger platelet aggregation as already shown for other aggregating agents such as ADP, 5-HT, thromboxane A_2 or thrombin. However, further in vivo studies in animal models of thrombosis with selective NK_1 receptor antagonists, will indicate if this property of neurokinins is related to some thrombotic event.

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