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Effects of BN 52063 and other agents inhibiting platelet-activating factor-induced contractile responses in rat portal vein

A. HELLEGOUARCH, M. AUGUET, F. CLOSTRE, P. BRAQUET, *I.H.B Research Labs, ZA de Courtaboeuf—1, avenue des Tropiques, 91952 Les Ulis Cedex, France*

Abstract—Platelet-activating factor (PAF-acether) is a potent agonist (EC50: $3{\cdot}2\times10^{-8}$ M) of isolated rat portal vein. BN 52063 (composed of BN 52020, BN 52021 and BN 52022; molar ratio 2:2:1) specifically inhibits PAF-acether (10^{-7} M) induced tone (IC50: $3{\cdot}9\times10^{-5}$ M). Salbutamol (IC50: $3{\cdot}1\times10^{-7}$ M), forskolin (IC50: $3{\cdot}1\times10^{-6}$ M) and theophylline (IC50: $2{\cdot}25\times10^{-4}$ M) are also effective in inhibiting PAF-acether-induced contractile responses and all excepting forskolin, show a certain specificity in this action. The basal myogenic activity of the rat portal vein is dose-dependently decreased by salbutamol (IC50: $1{\cdot}2\times10^{-7}$ M), forskolin (IC50: $2{\cdot}6\times10^{-6}$ M) and theophylline (IC50: $2{\cdot}3\times10^{-4}$ M) whereas BN 52063 has no effect. The data suggest that rat portal veins possess specific PAF-acether receptors sensitive to BN 52063 and that PAF-acether effects could be inhibited by compounds which can bypass these putative receptors and modulate cAMP levels.

Platelet-activating factor (PAF-acether, 1-*O*-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a potent mediator generated by platelets and neutrophils in inflammatory and allergic responses (Vargaftig et al 1981). However, several PAF-acether effects could be platelet and neutrophil independent such as the in-vivo increase of vascular permeability and hypotensive activity (Humphrey et al 1982; Page et al 1984), the in-vitro contractile response of guinea-pig lung parenchymal tissue and endothelium-dependent arterial relaxation (Stimler & O'Flaherty 1983; Lefort et al 1984; Vanhoutte & Houston 1985).

It has been shown that PAF-acether contracts the rat portal vein in-vitro (Santamaria et al 1983) and that this contraction is inhibited by the specific PAF-acether antagonist BN 52021 (ginkgolide B, Fig. 1) (Baranes et al 1986). BN 52063 (composed

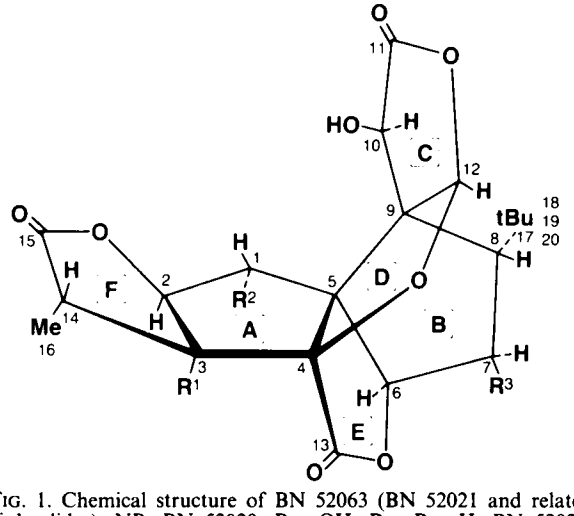

FIG. 1. Chemical structure of BN 52063 (BN 52021 and related ginkgolides). NB: BN 52020, $R_1=OH$, $R_2=R_3=H$; BN 52021, $R_1=R_2=OH$, $R_3=H$; BN 52022, $R_1=R_2=R_3=OH$.

of BN 52020, BN52021 and BN 52022, molar ratio 2:2:1) has been shown to inhibit PAF-acether induced platelet aggregation in different species (Braquet 1986) individually, BN 52021 being the most potent ginkgolide in this respect.

Correspondence to: P. Braquet, I.H.B. Research Labs, 17, avenue Descartes, 92350 Le Plessis-Robinson, France.

Increases in intracellular cAMP have also been shown to inhibit the aggregating action of PAF-acether (Bussolino & Camussi 1980; Vargaftig et al 1984). Therefore, using the above model, we have examined the pharmacological properties of BN 52063 in comparison with three drugs which indirectly modulate cyclic nucleotide levels: (i) a β_2-agonist, (ii) a phosphodiesterase inhibitor and (iii) forskolin which increases intracellular cAMP by activation of adenylate cyclase (Seamon & Daly 1981).

Methods

Male Sprague Dawley rats (200–250 g) were killed by cervical dislocation. The portal veins were opened longitudinally and strips (2–3 mm in width and 12–15 mm in length) suspended vertically under 0·5 g tension in a 20 mL tissue bath containing glucosed Krebs-Henseleit solution (mM) (NaCl: 118; KCl: 4·7; $MgSO_4$: 1·17; KH_2PO_4: 1·14; $CaCl_2$: 2·5; $NaHCO_3$: 25; glucose: 11) at 37°C, oxygenated with 95% O_2/5% CO_2, (pH = 7·4–7·5). The longitudinal muscle activity of the portal veins were measured isotonically using a Hall effect isotonic transducer (7006 Ugo Basile) coupled to a physiograph (Gemini II, Ugo Basile). Tissues were equilibrated for 1 h before use. Phentolamine (3×10^{-7} M) was added to the bath in experiments using salbutamol.

After the rest-period, the spontaneous myogenic activity of the portal veins was observed for 15 min; putative antagonists were then introduced into the bath. The agonists were added after maximum effect of antagonists on myogenic activity or after 30 min if no effects were observed. As PAF-acether completely desensitized the portal vein to restimulation by itself, only single doses per tissue were used. Four randomized preparations were tested in parallel and control experiments were performed under the same conditions.

Antagonists were thus tested for their ability to alter both basal myogenic activity and the agonist-induced rise in tonus. Agonist responses were measured as a change in basal tone of the preparation without taking into account the surmounting phasic activity (see Hicks 1983). Data are presented as mean ± s.e.m. The EC50 and IC50 values were calculated using linear regression (method of the least squares). ANOVA was used for statistical analysis of the results.

Drugs used. PAF-acether (Bachem.) was stored at −80°C in a 0·5% albumin normal saline stabilizing solution and diluted with Krebs-Henseleit buffer to obtain the experimental concentrations. BN 52063 (I.H.B) and forskolin (Calbiochem) were dissolved in dimethylsulfoxide (DMSO). Salbutamol (10^{-4} M) (Sigma) was dissolved in 0·1 M HCl and further dilutions made with deionized water. PGE_2 (Sigma) was initially dissolved in 33% ethanol in deionized water, further dilutions being made with the Krebs medium. Neither DMSO (maximum concentration 0·3%), HCl (maximum concentration 0·005%) nor ethanol (maximum concentration 0·015%) had any effect on the parameters studied. Carbachol; 5-hydroxytryptamine (5-HT), theophylline (Sigma) and phentolamine (Ciba-Geigy) were dissolved in deionized water.

Results

Effects of agonists. PAF-acether ($3 \times 10^{-9} - 3 \times 10^{-6}$ M) elicited a dose-dependent rise in tone of the portal vein (EC50 = $3{\cdot}2 \times 10^{-8}$ M; n = 3–9) and proved the most potent agonist tested. However, complete desensitization of the preparations to this phospholipid occurred after exposure to PAF-acether 10^{-7} M while the tissues were still responsive to other agonists (data not shown), necessitating the use of single doses of agonist throughout the study. In order to compare the four agonists on an equal basis, the dose that raised the tonus to a level approximately equal to the amplitude of the basal activity was used: PAF-acether: 10^{-7} M; carbachol: 10^{-6} M; 5-HT: 3×10^{-6} M and PGE_2: 10^{-5} M (Fig. 2).

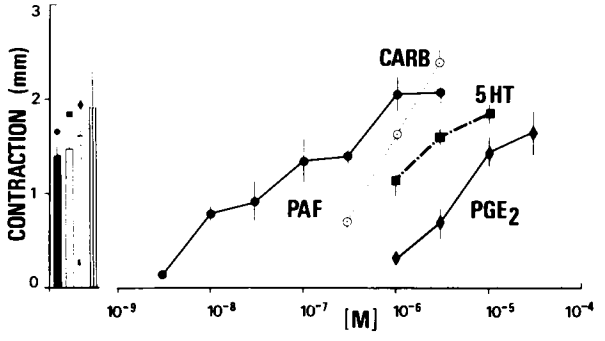

FIG. 2. Comparison of dose-dependent-tonus effects produced by the different agonists tested. The basal myogenic activities of preparations used for single doses of agonist giving comparable tonic effects are shown at the left of the graph: PAF-acether 10^{-7} M; 5-HT 3×10^{-6} M; PGE_2 10^{-5} M and carbachol (CARB) 10^{-6} M. Mean ± s.e.m. (n = 3–8).

Effects of antagonists. Salbutamol (IC50: $1{\cdot}2 \times 10^{-7}$ M, n = 3–15), forskolin (IC50: $2{\cdot}6 \times 10^{-6}$ M, n = 5–16) and theophylline (IC50: $2{\cdot}2 \times 10^{-4}$ M, n = 4–10) elicited dose-dependent inhibitions of the basal myogenic activity whereas BN 52063, up to 10^{-4} M, was without effect (Fig. 3).

All the drugs tested significantly attenuated the vascular response to PAF-acether (10^{-7} M): salbutamol IC50: $1{\cdot}2 \times 10^{-7}$ M, (n = 3–8); forskolin, IC50: $3{\cdot}1 \times 10^{-6}$ M, (n = 4–5); theophylline, IC50: $2{\cdot}25 \times 10^{-4}$ M, (n = 4–5); BN 52063, IC50: $3{\cdot}9 \times 10^{-5}$ M, (n = 5–6). BN 52063 and, to a lesser extent, salbutamol and theophylline (Fig. 4) demonstrated a certain specificity in their ability to inhibit PAF-acether induced tone whereas forskolin counteracted all agonists tested.

Discussion

PAF-acether (EC50: $3{\cdot}2 \times 10^{-8}$ M) increased the tone of rat portal vein and was thus about 100 times more potent than PGE_2 on this model. This effect occurred at doses similar to those needed to produce contractions in guinea-pig ileum or lung parenchymal tissues (Stimler et al 1981; Findlay et al 1981) or to induce human platelet aggregation (Vargaftig et al 1984).

BN 52021 and related compounds are specific inhibitors (Fig. 1) of PAF-acether induced platelet aggregation and lung strip contraction (Braquet 1986). In the present study, BN 52063 (composed of BN 52020, BN 52021 and BN 52022; molar ratio 2:2:1) counteracted the vascular contraction induced by PAF-acether. But, in this respect, BN 52063 (IC50: $3{\cdot}9 \times 10^{-5}$ M) was slightly less potent than BN 52021 alone (IC50: $1{\cdot}6 \times 10^{-5}$ M; Baranes et al 1986).

In the rat portal vein, PAF-acether induced tone desensitized specifically the preparation to restimulation by PAF-acether itself and was selectively inhibited by BN 52063; thus it can be postulated that this vessel possesses specific PAF-acether receptors sensitive to ginkgolides.

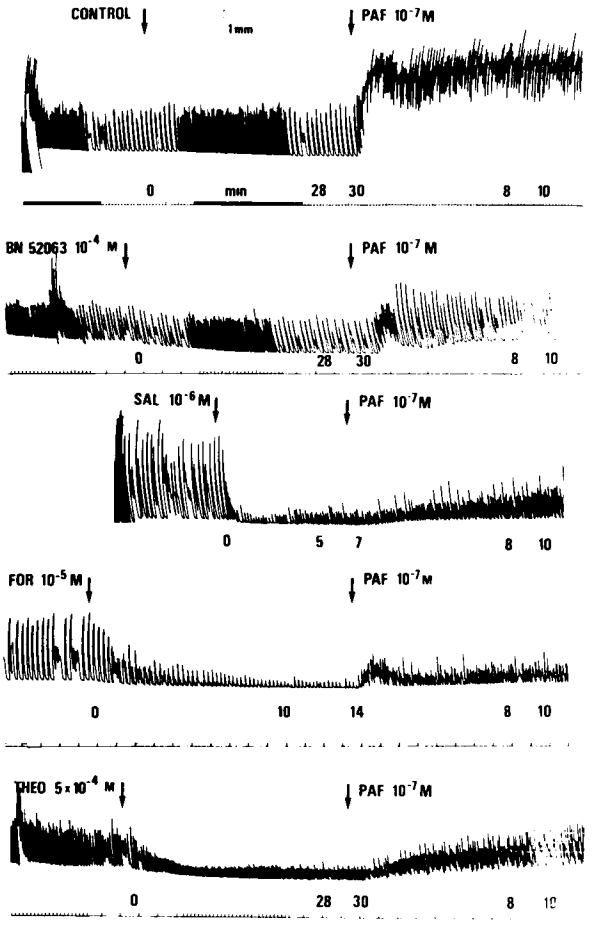

FIG. 3. Representative recording of PAF-induced contractile response in rat portal vein and its inhibition by BN 52063 (10^{-4} M); salbutamol (SAL, 10^{-6} M); forskolin (FOR, 10^{-5} M) and theophylline (THEO, 5×10^{-4} M); Note that only BN 52063 does not modify the basal myogenic activity.

It could be argued that BN 52063 directly inhibits the contactile process induced by PAF-acether but this hypothesis is not valid because (i) BN 52063 acts specifically against PAF-acether as opposed to the other agonists tested and (ii) does not modify the basal myogenic activity of the rat portal vein which is extremely sensitive to calcium modulators (i.e.: calcium entry blockers and phosphodiesterase inhibitors) (Berti et al 1970; Vanhoutte 1982). Calcium channel blockers such as D600 and diltiazem have been shown to inhibit the spontaneous myogenic activity of the portal vein without any appreciable change of the PAF-acether-induced tone (Baranes et al 1986) whereas both these parameters are antagonized by theophylline and forskolin.

Salbutamol (a β_2-agonist), forskolin (an adenylate cyclase activator) and theophylline (a phosphodiesterase inhibitor) are known to increase intracellular cAMP which in turn mediates the subsequent cellular events leading to relaxation (Jackson & McNeill 1985; Seamon & Daly 1981). These three products decreased myogenic activity and agonist-induced tone and were all more effective in inhibiting PAF-acether effects than PGE_2, 5-HT or carbachol, with salbutamol showing the highest selectivity towards PAF-acether.

These results demonstrate that increasing intracellular cAMP inhibits PAF-acether effects, as has been previously shown in platelet aggregation studies (Bussolino & Camussi 1980; Vargaftig et al 1984).

It has been shown that relatively high concentrations (10^{-6}–10^{-4} M) of PAF-acether induce an endothelium-dependent

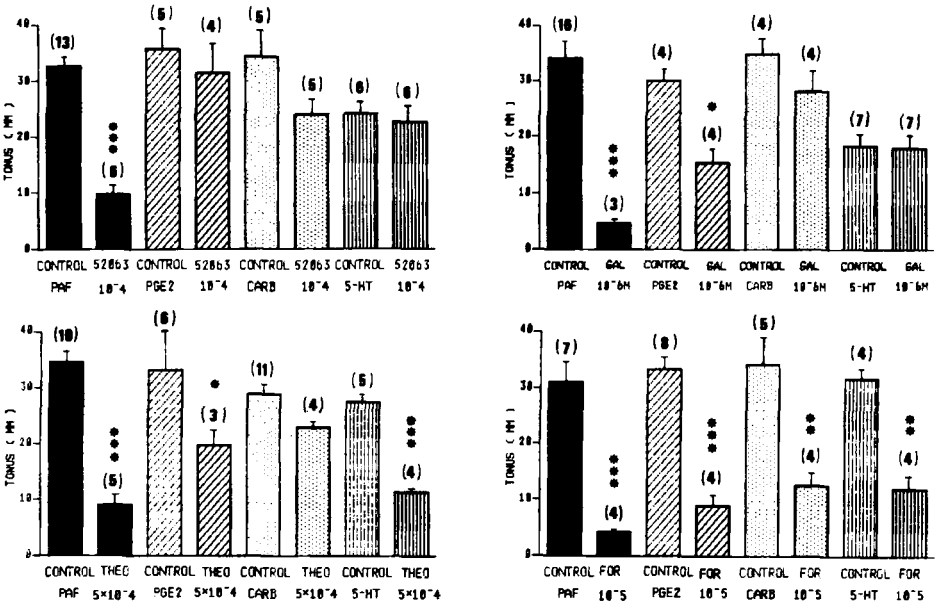

FIG. 4. Comparison of effects of BN 52063 (10^{-4} M, upper, left), salbutamol (SAL, 10^{-6} M, upper, right), theophylline (THEO, 5×10^{-4} M, lower, left) and forskolin (FOR, 10^{-5} M, lower right) on PAF-acether, PGE_2, carbachol (CARB) and 5-HT induced tonus. Mean ± s.e.m. (n). Vertical axis is expressed into graphic recording mm. Sensitivity scale 1 mm real: 20 mm graphic. * $P<0.05$; ** $P<0.01$; *** $P<0.001$ for comparison with respective control.

relaxation of rat aorta (Cervoni et al 1983; Kamitani et al 1984) and canine femoral and coronary arteries (Vanhoutte & Houston 1985). In this latter model, PAF-acether induced vasodilatation was not inhibited by the PAF-acether antagonist, CV 3988 (*RS*)-2-methoxy-3-(octadecylcarbamoyloxy)propyl 2-(3-thiazolio)ethyl phosphate. It thus seems that this endothelium-dependent relaxation could be due to a detergent or ionophoretic-like action as already demonstrated with acetal plasmalogens (Braquet et al 1987).

In conclusion, the present data clearly demonstrate that, unlike arterial vessels, the rat portal vein is contracted by PAF-acether. This direct vascular effect may be due to the stimulation of specific PAF-acether receptors. The rat portal vein, with its inherent myogenic activity, is a sensitive model able to distinguish between specific PAF-acether antagonists acting at the putative receptor level and non-specific modulators of PAF-acether activity (i.e., β_2-agonist, adenyl-cyclase activator, phosphodiesterase inhibitors and calcium modulators).

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