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Involvement of K^+ channel permeability changes in the L-NAME and indomethacin resistant part of adenosine-5′-O-(2-thiodiphosphate)-induced relaxation of pancreatic vascular bed

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1 We have previously demonstrated that adenosine-5′-O-(2-thiodiphosphate) (ADPβS), a potent P2Y-purinoceptor agonist, relaxed pancreatic vasculature not only through prostacyclin (PGI_2) and nitric oxide (NO) release from the endothelium but also through other mechanism(s). In this study, we investigated the effects of an inhibitor of the Na^+/K^+ pump, of ATP-sensitive K^+ (K_{ATP}) channels and of small (SK_{Ca}) or large (BK_{Ca}) conductance Ca^{2+}-activated K^+ channels. Experiments were performed at basal tone and during the inhibition of NO synthase and cyclo-oxygenase.

2 In control conditions, ADPβS (15 μM) induced an initial transient vasoconstriction followed by a progressive and sustained vasodilatation. In the presence of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 200 μM) the transient vasoconstriction was reversed into a one minute vasodilator effect, which was then followed by a progressive and sustained vasodilatation similar to that observed with ADPβS alone. The addition of indomethacin (10 μM) did not significantly modify the profile of ADPβS-induced vasodilatation.

3 Ouabain (100 μM) decreased basal pancreatic flow rate and did not modify ADPβS-induced relaxation. This inhibitor of the Na^+/K^+ pump increased the pancreatic vasoconstriction induced by L-NAME or by the co-administration of L-NAME and indomethacin. Ouabain did not modify either the L-NAME or the L-NAME/indomethacin resistant part of the ADPβS vasodilatation.

4 The K_{ATP} inhibitor tolbutamide (185 μM) did not significantly modify basal pancreatic flow rate and ADPβS-induced relaxation. This inhibitor which did not change L-NAME-induced vasoconstriction, significantly diminished the L-NAME resistant part of ADPβS-induced vasodilatation. Tolbutamide intensified the vasoconstriction induced by the co-administration of L-NAME and indomethacin. In contrast, the L-NAME/indomethacin resistant part of ADPβS vasodilatation was not changed by the closure of K_{ATP}.

5 The SK_{Ca} inhibitor apamin (0.1 μM) did not significantly change pancreatic vascular resistance whatever the experimental conditions (in the absence or in presence of L-NAME or L-NAME/indomethacin). In the presence of L-NAME, the closure of SK_{Ca} channels changed the one minute vasodilator effect of ADPβS into a potent vasoconstriction and thereafter modified only the beginning of the second part of the L-NAME-resistant part of the ADPβS-induced vasodilatation. In contrast, the L-NAME/indomethacin resistant part of ADPβS-induced relaxation remained unchanged in the presence of apamin.

6 Charybdotoxin (0.2 μM), an inhibitor of BK_{Ca}, increased pancreatic vascular resistance in the presence of L-NAME/indomethacin. In the presence of L-NAME, the closure of BK_{Ca} channels reversed the one minute vasodilator effect of ADβPS into a potent vasoconstriction and drastically diminished the sustained vasodilatation. In contrast the L-NAME/indomethacin resistant part of ADPβS-induced relaxation was not modified by the presence of charybdotoxin. Under L-NAME/indomethacin/charybdotoxin/apamin infusions, ADPβS evoked a drastic and transient vasoconstriction reaching a maximum at the second minute, which was followed by a sustained increase in the flow rate throughout the ADPβS infusion. The maximal vasodilator effect of ADPβS observed was not modified by the addition of apamin.

7 The results suggest that the L-NAME-resistant relaxation induced by ADPβS in the pancreatic vascular bed involves activation of BK_{Ca}, K_{ATP} and to a lesser extent of SK_{Ca} channels, but the L-NAME/indomethacin resistant part of ADPβS-induced relaxation is insensitive to the closure of K_{ATP}, SK_{Ca} and BK_{Ca} channels.

Keywords: Pancreatic vascular bed; ADPβS; potassium channels; P2-receptors

Introduction

Purine nucleotides are physiological regulators of vascular tone acting both as an intraluminal and as a neuronal messenger (Burnstock, 1993). P2X receptors inducing vasoconstriction and P2Y receptors inducing vasodilatation are both present on pancreatic vessels (Hillaire-Buys *et al*., 1991). P2Y receptors are generally located in vascular endothelial cells (Olsson & Pearson, 1990; Burnstock *et al*., 1994); but, a smooth muscle localization has also been demonstrated (Mathieson & Burnstock, 1985; Brizzolara & Burnstock, 1991; Strobaeck *et al*., 1996). P2Y receptors belong to the family of receptors with 7 transmembrane – spanning domains and their stimulation generally leads to the activation of phospholipase C, formation of inositol 1,4,5-triphosphate (IP_3) and subsequent release of Ca^{2+} from internal stores (Lustig *et al*., 1992; Barnard *et al*., 1994). In addition, it has

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been found that the activation of some P2 receptors induces stimulation of adenylyl cyclase in vascular smooth muscle and phaeochromocytoma cells (Tada *et al*., 1992; Yakushi *et al*., 1996). A P2Y vasodilatator effect occurs as a consequence of the release of both prostacyclin (PGI_2) and nitric oxide (NO) or related molecules (Boeynaems & Pearson, 1990).

We have recently shown that the relaxation of pancreatic vessels induced by adenosine-5′-O-(2-thiodiphosphate) (ADPβS), a non hydrolyzable and selective P2Y agonist (Welford *et al*., 1987; Watson & Girdlestone, 1996) involves other mechanism(s) than NO and PGI_2 release (Saïag *et al*., 1996). The existence of another relaxant factor released by endothelial cells has been proposed in acetylcholine – or histamine – induced vasodilatation; this factor is called 'endothelium derived hyperpolarizing factor' (EDHF) (Chen *et al*., 1988; Chen & Suzuki, 1989) and may explain the resistance of some endothelium-dependent responses to inhibitors of nitric oxide synthase and of cyclo-oxygenase. The EDHF-induced hyperpolarization has been shown to involve an increase in membrane conductance, which appears primarily to reflect an increase in the movement of K^+ ions (Garland *et al*., 1995). Depending on the preparation being studied, an activation of the Na^+/K^+ pump (Félétou & Vanhoutte, 1988) and/or a direct opening of ATP-dependent (Garland & McPherson, 1992) or Ca^{2+}-dependent K^+ channels (Adeagbo & Triggle, 1993) have been proposed to explain the EDHF-induced hyperpolarization. EDHF-induced hyperpolarization seems to be predominant in small resistance vessels rather than in large arteries (Garland *et al*., 1995). It can be hypothesized that this mechanism may play a role in ADPβS-induced relaxation of pancreatic vessels. For this purpose, we have investigated the effect of an inhibitor of the Na^+/K^+ pump (ouabain), of an ATP-sensitive K^+ (K_{ATP}) channel blocker (tolbutamide), of a small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channel blocker (apamin) and of a large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel blocker (charybdotoxin). Experiments were performed at basal tone and under inhibition of either NO synthase alone or NO synthase plus cyclo-oxygenase by N^{ω}-nitro-L-arginine methyl ester (L-NAME) and indomethacin, respectively.

Methods

The experiments were carried out on the isolated perfused pancreas of the rat, according to the technique previously described by Loubatières *et al*. (1969). Male Wistar rats weighing 300 – 350 g received 60 mg kg^{-1} sodium pentobarbitone by i.p. injection. The pancreas was totally isolated from all neighbouring tissues and organs; it was perfused through its own arterial system with a Krebs-Ringer bicarbonate buffer (composition in mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5) containing bovine serum albumin (2 g l^{-1}) and 8.3 mM glucose. A mixture of O_2 (95%) and CO_2 (5%) was bubbled at atmospheric pressure. The pH of the solution was 7.35. The preparation was maintained at 37.5°C. Each organ was perfused at a constant pressure (in the range 40 – 50 cmH_2O), selected so as to obtain a flow rate of 2.5 ml min^{-1} at the start of the experiment. Any change in pancreatic vascular bed resistance due to drug administration resulted in a change in the flow rate; the latter was measured by collecting each sample in a graduated tube for 1 min, the error of the measure being 0.05 ml maximum.

We carried out a kinetic study for 105 min which was divided into four periods: (1) a 45 min equilibration period (0 – 45 min of organ perfusion) in which basal flow rates were measured twice, at 30 and 45 min before any drug administration; (2) the next 15 min (45 – 60 min of organ perfusion) in which were added L-NAME (200 μM), indomethacin (10 μM), ouabain (100 μM), tolbutamide (185 μM), apamin (0.1 μM), charybdotoxin (0.2 μM) or various combinations of these different inhibitors; (3) in the third period (60 – 90 min) ADPβS (15 μM) was infused in the absence of any other drug (control experiments) or in the presence of the inhibitor(s); (4) the final period (90 – 105 min) allowed us to control the reversibility of flow rate changes.

Indomethacin was used at a concentration of 10 μM since at this concentration this substance acts as inhibitor of cyclo-oxygenase (Gambone *et al*., 1997; Miralpeix *et al*., 1997). Higher concentrations can act not only by inhibition of the cyclo-oxygenase pathway but also by inhibition of Ca^{2+}-dependent biological systems in a non-specific manner (Gorog & Kovac, 1970; Northover, 1971; Lewis & Whittle, 1977). L-NAME was used at 200 μM, a concentration which elicited a stable and maximum fall in the flow rate in basal tone. Higher concentrations of L-NAME did not induce a larger fall of flow rate in our preparation (Gross *et al*., 1995).

The graphs represent the kinetics flow rate. The flow rate was measured every minute during the first 5 min, then 8, 10, 15, 20 and 30 min after the addition of ADPβS.

Statistical analysis

The results are expressed as means ± s.e.mean in absolute values and in some cases as a percentage of the 60 min value (just before ADPβS infusion). Analysis of variance was applied, followed by the multiple comparison test (Zar, 1974).

Drug used

Adenosine-5′-O-(2-thiodiphosphate)-lithium salt, apamin, indomethacin, L-NAME, ouabain, tolbutamide, were from Sigma (U.S.A.); synthetic charybdotoxin was from Latoxan (Rosans, France). All drugs were directly dissolved in the physiological solution except indomethacin and tolbutamide; these drugs were first dissolved in ethanol and in 1 N sodium hydroxide respectively. Both drugs were then diluted in the physiological solution (final concentration of ethanol: 0.0008%; final concentration of sodium hydroxide: 0.0002%). At these final concentrations, the solvents have no effect on pancreatic vascular resistance.

Results

The effects of K^+ permeability inhibitors (ouabain, tolbutamide, apamin and charybdotoxin) were studied in three conditions: (1) at basal tone; (2) in the presence of L-NAME; (3) in the presence of L-NAME plus indomethacin. The results were analysed in two parts, the effects of K^+ permeability inhibitors *per se* and on the ADPβS-induced changes in pancreatic vascular resistance.

Effects of K^+ permeability inhibitors per se

At basal tone, ouabain (100 μM) induced a significant decrease in the pancreatic flow rate which reached a maximum of −16%, five min after the beginning of the infusion (Figure 1a). In the presence of tolbutamide (185 μM) or charybdotoxin (0.2 μM), a trend to vasoconstriction was observed, although not significant (Figures 2a and 4a). In contrast, apamin

(0.1 μM) elicited a slight but not significant increase in the pancreatic flow rate (Figure 3a).

As previously shown in our preparation, L-NAME (200 μM) evoked an immediate, stable and potent vasoconstriction (Figure 1b) which was not modified by the addition of indomethacin (10 μM) (Figure 1c), this latter inhibitor being without effect at basal tone (Saïag *et al.*, 1996). Ouabain in these conditions enhanced the decrease of flow rate observed in

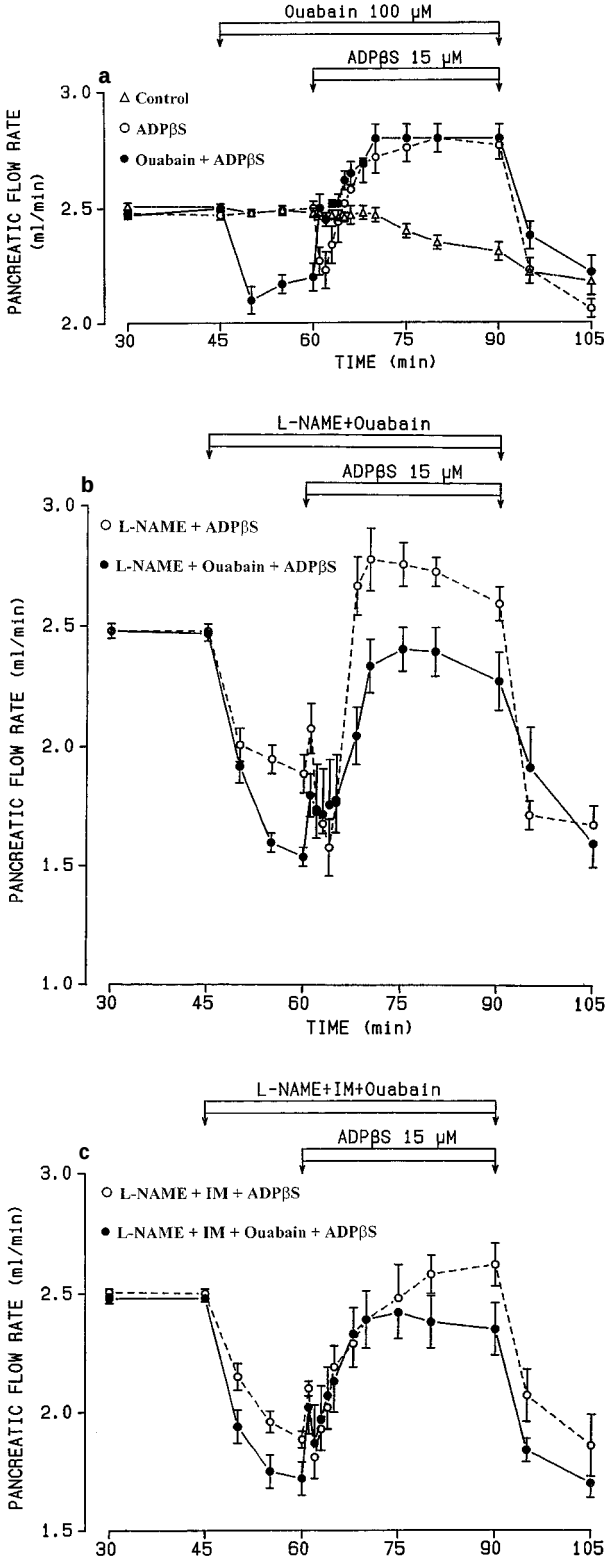

Figure 1 Effects of ouabain (100 μM) on the pancreatic vascular resistance modifications induced by ADPβS in three conditions: (a) at basal tone; (b) in the presence of L-NAME (200 μM); (c) in the presence of L-NAME + indomethacin (IM; 10 μM). Each point represents the mean and vertical lines s.e.mean of six experiments. Controls perfused in the absence of any drug are indicated in graph (a).

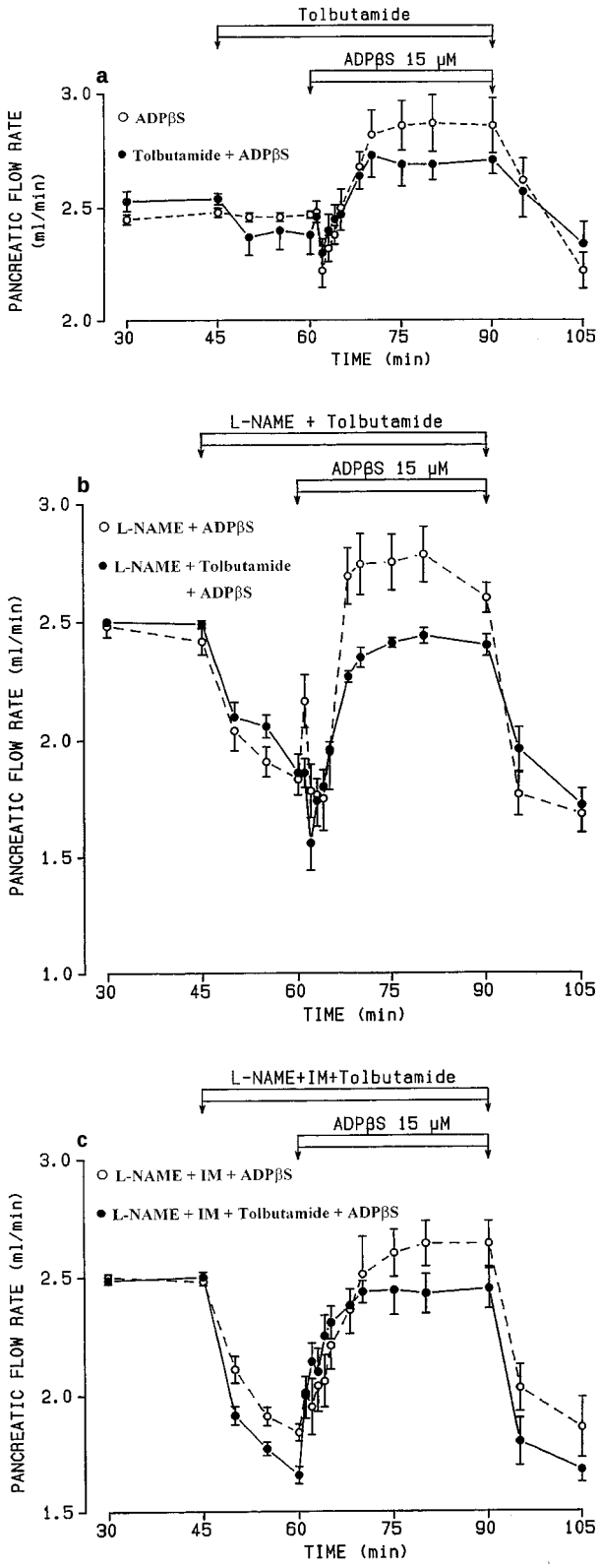

Figure 2 Effects of tolbutamide (185 μM) on the pancreatic vascular resistance modifications induced by ADPβS in three conditions: (a) basal tone; (b) in the presence of L-NAME (200 μM); (c) in the presence of L-NAME + indomethacin (IM; 10 μM). Each point represents the mean and vertical lines s.e.mean of seven experiments.

the presence of L-NAME or L-NAME/indomethacin (Figure 1b and c). The tendency of tolbutamide or charybdotoxin to decrease the flow rate was not observed in the presence of L-NAME (Figures 2b and 4b), but appeared in the presence of L-NAME/indomethacin (Figures 2c and 4c). As for the slight vasodilatation elicited by apamin, it disappeared in presence of L-NAME/indomethacin (Figure 3c).

Effects of K^+ permeability inhibitors on ADPβS induced vasodilatation

In control experiments performed in the presence of glucose 8.3 mM alone, the vascular flow rate was not significantly modified during the entire 105 min of the experiments, although there was a slight and progressive decrease (Figure 1a). ADPβS 15 μM induced a dual effect on pancreatic vascular flow rate: an initial transient vasoconstriction, significant only at the second min ($P<0.01$ versus 45 min value), followed by a progressive vasodilatation within the first 10 min (first period) which reached a plateau for the following 20 min (second period) (Figures 1a, 2a, 3a and 4a).

At basal tone In the presence of ouabain (100 μM) the slight vasoconstrictor effect of ADPβS disappeared and the ADPβS-induced vasodilatation was not significantly modified by this inhibitor of the Na^+/K^+ pump (Figure 1a). Tolbutamide (185 μM) did not significantly change the ADPβS vascular effects (Figure 2a). Apamin (0.1 μM) did not change the slight vasoconstrictor effect of ADPβS; it did not significantly modify the vasodilatation induced by the purinoceptor agonist (Figure 3a). Charybdotoxin (0.2 μM) significantly increased the slight vasoconstrictor effect of ADPβS at one minute ($P<0.001$) (Figure 4a).

In the presence of L-NAME The initial transient vasoconstriction observed with ADPβS in control conditions (without any other drug) was reversed to a transient but not significant increase in pancreatic flow rate for one minute. This one minute vasodilator effect of ADPβS was followed by both a change in the flow rate and a maximal vasodilatation similar to that observed with ADPβS alone, confirming previous results (Saïag *et al.*, 1996).

In the presence of ouabain, the L-NAME-resistant part of ADPβS-induced relaxation seemed to be diminished when the results were expressed in absolute values (Figure 1b). However, since ouabain *per se* increased L-NAME-induced vasoconstriction, the maximal vasodilator effect of ADPβS observed between the 70th to the 90th min did not differ in the absence or in the presence of ouabain ($+47\pm3\%$ versus $+57\pm4\%$ for experiments performed in the absence or in the presence of ouabain respectively).

Tolbutamide which did not modify the L-NAME-induced vasoconstriction, abolished the one minute vasodilator effect of ADPβS and significantly diminished the sustained second period of the L-NAME-resistant part ADPβS-induced relaxation however the results were expressed, in absolute values or as a percentage of the 60 min value ($+53\pm9\%$ versus $+33\pm8\%$ for experiments performed in the absence or in the presence of tolbutamide respectively, $P<0.01$) (Figure 2b).

Apamin changed the one minute vasodilator effect of ADPβS into a potent vasoconstriction which peaked to $-33\pm4\%$ at the second minute; and consequently significantly modified only the beginning of the second period of L-NAME resistant part of ADPβS-induced vasodilatation ($P<0.01$) (Figure 3b).

Charybdotoxin reversed the one minute vasodilator effect of ADPβS into a drastic vasoconstriction which peaked to $-35\pm4\%$ at the second minute; thereafter the second period of the L-NAME resistant part of ADPβS-induced vasodilata-

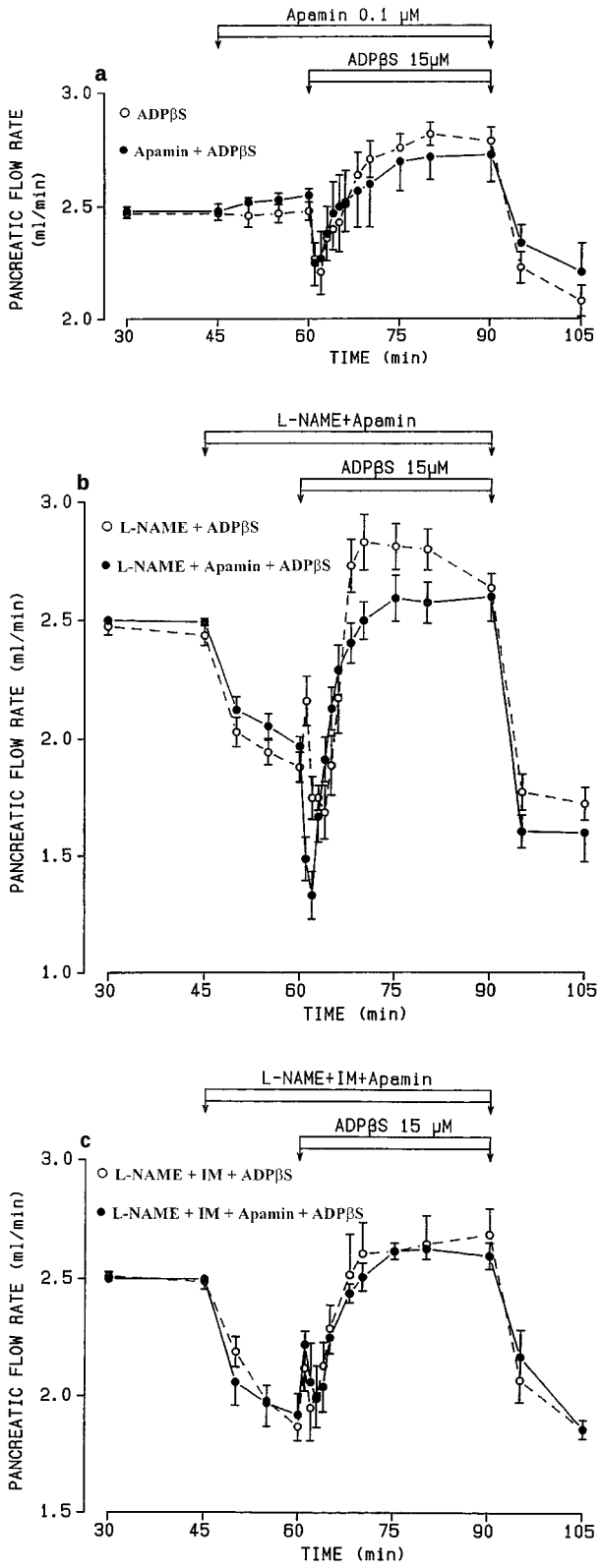

Figure 3 Effects of apamin (0.1 μM) on the pancreatic vascular resistance modifications induced by ADPβS in three conditions: (a) at basal tone; (b) in the presence of L-NAME (200 μM); (c) in the presence of L-NAME + indomethacin (IM; 10 μM). Each point represents the mean and vertical lines s.e.mean of seven experiments.

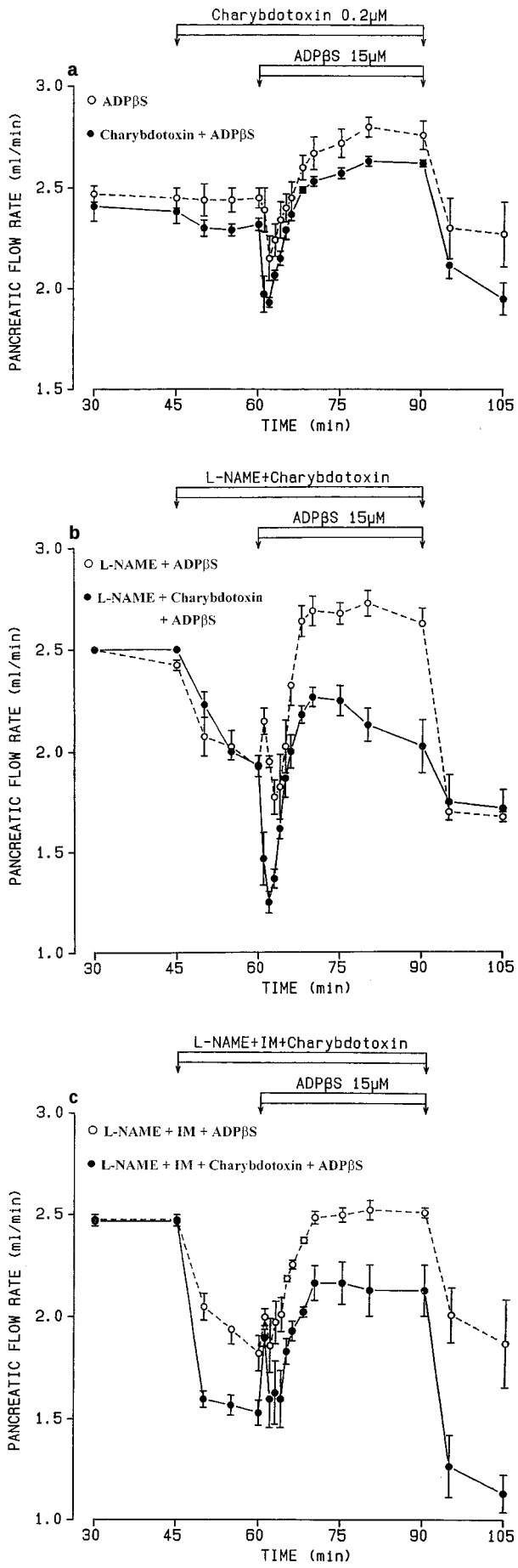

Figure 4 Effects of charybdotoxin (0.2 μM) on the pancreatic vascular resistance modifications induced by ADPβS in three conditions: (a) at basal tone; (b) in the presence of L-NAME (200 μM); (c) in the presence of L-NAME + indomethacin (IM; 10 μM). Each point represents the mean and vertical lines s.e.mean of five experiments.

tion was greatly diminished (the maximal vasodilator effect of ADPβS observed between 70th to the 90th being $+42\pm4\%$ versus $+10\pm3\%$ in the absence and presence of charybdotoxin, respectively) ($P<0.001$) (Figure 4b).

The addition of indomethacin Indomethacin did not significantly modify either the one minute vasodilator effect of ADPβS observed in the presence of L-NAME alone or the L-NAME-resistant part of ADPβS-induced vasodilatation during the second period, as previously described (Saïag *et al*., 1996). None of the inhibitors tested diminished the one minute vasodilator effect of ADPβS. Ouabain, tolbutamide and apamin did not change significantly the L-NAME/indomethacin resistant part of ADPβS-induced relaxation during the second period. In the presence of charybdotoxin, the L-NAME/indomethacin resistant part of ADPβS-induced relaxation seemed to be diminished when the results were expressed in absolute values (Figure 4c). However, since charybdotoxin *per se* increased L-NAME/indomethacin-induced vasoconstriction, the maximal vasodilator effect of ADPβS observed between the 70th to the 90th minute did not differ in the absence or presence of charybdotoxin ($+39\pm5\%$ versus $+39\pm4\%$, respectively). In another set of experiments, we tested the effects of the combination of charybdotoxin plus apamin on the L-NAME/indomethacin resistant part of ADPβS-induced relaxation (Figure 5). The vasoconstriction observed in the presence of L-NAME/indomethacin and charybdotoxin was slightly diminished by the addition of apamin. In these experimental conditions, ADPβS evoked a drastic and transient vasoconstriction reaching a maximum of $-59\pm8\%$ at the second minute, which was followed by a sustained increase in the flow rate throughout the ADPβS infusion (Figure 5). The maximal vasodilator effect of ADPβS observed between the 70th to the 90th min was not modified by the addition of apamin ($+39\pm4\%$ versus $+34\pm5\%$ for experiments performed in the absence or presence of apamin, respectively). After the addition of drugs had ceased the flow rate returned to values comparable to those observed before ADPβS infusion.

Discussion

This study shows that K^+ permeability inhibitors modify in a different way basal and P2 purinoceptor-induced relaxation of pancreatic vascular bed. Furthermore, the effects of these inhibitors are changed according to the experimental conditions i.e. blockade of NO synthase alone or blockade of NO synthase and cyclo-oxygenase.

Stimulation of the electrogenic Na^+/K^+ pump has been suggested to mediate endothelium-dependent hyperpolarization induced by acetylcholine (Félétou & Vanhoutte, 1988; Brayden & Wellman, 1989; Adeagbo & Malik, 1990a; Kitagawa *et al*., 1994). In this study, we showed that ouabain decreases pancreatic flow rate at basal tone. Thus, Na^+/K^+ exchange may play a role in maintaining a vasodilatator tone in our preparation. In rat isolated perfused mesenteric artery, ouabain infusion caused a transient increase in perfusion pressure (Adeagbo & Malik, 1991). The $[Na^+]_i$ increase can result from the inhibition of the pump and may accelerate Ca^{2+} influx leading to a depolarization of vascular cells

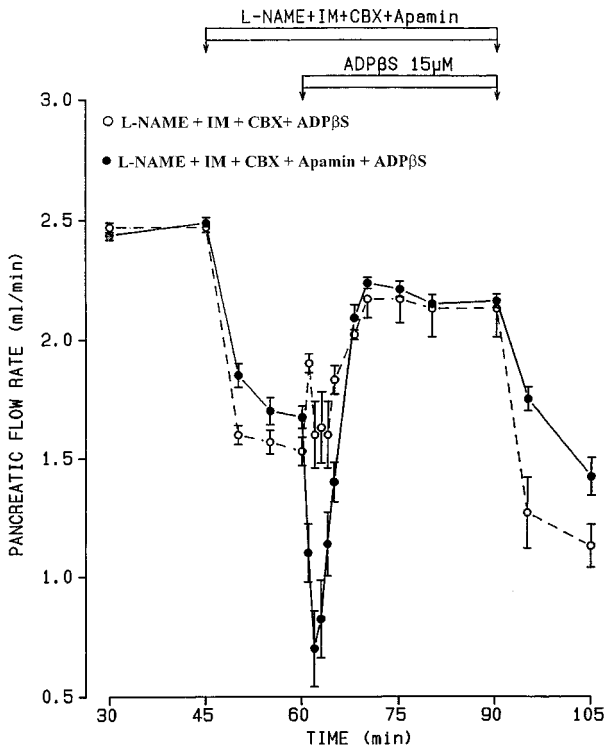

Figure 5 Effects of the combination of charybdotoxin (CBX) and apamin on the L-NAME/indomethacin (IM) resistant part of ADPβS-induced relaxation. Each point represents the mean and vertical lines s.e.mean of five experiments.

(Kuriyama *et al*., 1995). The vasoconstrictor effect of ouabain might also be due to the inhibition of an endogenous vasodilatator agent such as PGI_2. This is unlikely since the PGI_2 vasodilatator effect has been described to be insensitive to ouabain (Adeagbo & Malik, 1990b) and the blockade of cyclo-oxygenase by indomethacin did not change basal pancreatic resistance (Saïag *et al*., 1996). The vasoconstrictor effect of ouabain *per se* seems to be independent of NO synthase and the cyclo-oxygenase pathways, since it was not significantly modified in presence of L-NAME and indomethacin. With regard to the effect of ouabain on ADPβS-induced changes in pancreatic vascular resistance, we only observed the disappearance of the transient vasoconstriction induced by this purinoceptor agonist. It must be pointed out that this transient and slight vasoconstrictor effect of ADPβS is more or less prominent according to the experimental conditions (Hillaire-Buys *et al*., 1992; Saïag *et al*., 1996). ADPβS has been previously demonstrated to be a potent purinoceptor agonist inducing PGI_2 release from bovine endothelial cells (Lustig *et al*., 1992) and we have shown that the first period of ADPβS-induced vasodilatation was inhibited by indomethacin, suggesting the involvement of PGI_2 release (Saïag *et al*., 1996). On endothelial cells, the PGI_2 release induced by purine nucleotides was increased in the presence of ouabain (Boeynaems & Ramboer, 1989) and such a mechanism could support the rapid onset of ADPβS-induced vasodilatation occurring in the presence of ouabain. The present findings showing that the vasodilator action of ADPβS during the second period is not modified by ouabain, whatever the experimental conditions (in absence or presence of L-NAME or L-NAME plus indomethacin), suggests the existence of a mechanism other than Na^+/K^+ exchange. In some other preparations, ouabain failed to modify the vasodilatator effect of acetylcholine (Suzuki, 1988; Chen *et al*., 1991) and even, like our results, when NO synthase and cyclo-oxygenase were inhibited (Zygmunt & Högestätt, 1996).

Opening of K_{ATP} channels has also been shown to be involved in the hyperpolarization underlying vasodilatation evoked by acetylcholine (Standen *et al*., 1989; Brayden, 1990; Plane *et al*., 1995). However, the mechanism behind the hyperpolarization observed with acetylcholine may depend on the origin of the vessels. Thus, K_{ATP} channels are not involved in the hyperpolarization induced by acetylcholine in rat mesenteric arteries (McPherson & Angus, 1991; Garland & McPherson, 1992) and by acetylcholine and histamine in the rat perfused mesenteric arterial bed (Adeagbo & Triggle, 1993). Our results show that the blockade of K_{ATP} channels tended to diminish basal pancreatic flow rate. Since nitric oxide has been previously shown to activate K_{ATP} channels (Murphy & Brayden, 1995), a basal release of NO (Saïag *et al*., 1996) may offset the vasoconstrictor effect of tolbutamide. The fact that this vasoconstrictor effect of tolbutamide disappeared in presence of L-NAME and reappeared when indomethacin is added, also suggests a compensatory mechanism linked to an increased effect of vasodilatator prostanoïds when NO synthase is blocked. It has been shown that NO may inhibit the cyclo-oxygenase pathway (Lamontagne *et al*., 1992; Swierkosz *et al*., 1995). With regard to the effects of tolbutamide on ADPβS-induced changes in pancreatic vascular resistance, the vasodilator effect of ADPβS was diminished by the inhibitor of K_{ATP} only in experiments performed in the presence of L-NAME alone. Since the hyperpolarization induced by prostacyclin and its stable analogue iloprost is abolished by glibenclamide, another inhibitor of K_{ATP} channels (Jackson *et al*., 1993; Corriu *et al*., 1996), we propose that PGI_2 release could play a role, at least in part, in ADPβS-induced relaxation but only when NO synthase is blocked.

Opening of calcium-activated K^+ channels has also been shown to be involved in vascular hyperpolarization (Bolotina *et al*., 1994; Murphy & Brayden, 1995). This hyperpolarization could be a consequence of exogenous NO administration or EDHF pathway activation. Our results show that the large conductance calcium-activated K^+ channels (BK_{Ca}) tended to decrease basal pancreatic flow rate, whereas the blockade of small conductance calcium-activated K^+ channels (SK_{Ca}) tended to increase the basal flow rate. In the presence of L-NAME alone, the one minute vasodilator effect of ADPβS was reversed into a potent vasoconstriction by apamin or charybdotoxin (Figures 3b and 4b). Such an effect disappeared when indomethacin is added (Figures 3c and 4c). Furthermore, a potent vasoconstriction was still observed during the first five minutes of ADPβS infusion when apamin and charybdotoxin were combined in the presence of L-NAME/indomethacin (Figure 5). This suggests that the one minute vasodilator effect of ADPβS involves either SK_{Ca} or BK_{Ca} channels, or another type of K^+ channel inhibited by apamin and charybdotoxin in a synergistic manner. Such an interaction of these two toxins with a common site or membrane protein has been recently proposed (Gebremedhin *et al*., 1996; Petersson *et al*., 1997). As for the L-NAME-resistant part of ADPβS-induced relaxation during the second period, a small inhibitory effect of apamin was observed (Figure 3b) whereas charybdotoxin drastically diminished this L-NAME resistant response (Figure 4b). Since both effects of these toxins were abolished when indomethacin was added, we propose that BK_{Ca} and SK_{Ca} channels, at least to some extent, could be opened by vasodilator prostanoïds in the presence of an inhibitor of NO synthase. The NO/prostanoid-independent relaxation induced by acetylcholine has been shown to be inhibited by apamin (Adeagbo & Triggle,

1993; Garcia-Pascual *et al*., 1995) and in some preparations, a combination of both apamin and charybdotoxin is necessary to suppress acetylcholine-induced relaxation (Zygmunt & Högestätt, 1996; Petersson *et al*., 1997) or hyperpolarization (Corriu *et al*., 1996). Surprisingly, apamin or charybdotoxin alone as well as charybdotoxin used in combination with apamin were unable to prevent the relaxation induced by ADPβS during the second period when NO synthase and cyclo-oxygenase were blocked. This suggests that the L-NAME/indomethacin independent part of P2Y-induced pancreatic relaxation does not involve SK_{Ca} or BK_{Ca} channels. Furthermore, these findings suggest that the K^+ channels involved in vascular relaxation could be differently involved according to the agonist used, the experimental conditions and the vascular system studied. The fact that ADPβS is always able to induce vascular relaxation allowed us to suspect other mechanism(s) involved in P2Y-induced pancreatic relaxation. Since P2Y-induced pancreatic relaxation is, at least in part, preserved in noxious conditions (blockade of NO and PGI_2 pathways plus closure of Ca^{2+} activated K^+ channels), it is proposed that P2Y-agonists may be highly interesting substances with clinical relevance in pathological vasospasm and ischaemia.

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