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Sodium nitroprusside regulates the relaxation of the longitudinal muscle in the gut

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Nitric oxide (NO) has been shown to mediate nonadrenergic-noncholinergic relaxation in gastrointestinal (GI) smooth muscle cells. As GI smooth muscles relaxations are partly dependent on NO, we decided to investigate the effect of sodium nitroprusside (SNP) on the longitudinal muscle contraction of the isolated guinea pig ileum. Increasing concentrations of SNP (10^{-10}M, 10^{-9}M, 10^{-8}M, 10^{-7}M, 10^{-6}M and 10^{-5}M) reduced ileum contractions stimulated by electrical stimulation (ES) (8–76%; $p < 0.05$) and by acetylcholine (Ach) (23–62%; $p < 0.05$) significantly and in a concentration-dependent manner. Furthermore, treatment with an inhibitor of the soluble guanylate cyclase, methylene blue (10 mM), antagonized significantly the relaxing effect of SNP (0–39%; $p < 0.05$, $p < 0.01$, $p < 0.001$ for ES- and 4–27%; $p < 0.05$ for Ach-induced contractions). The results show that treatment with 1 μM manganese-containing superoxide dismutase (MnSOD) and 10 μM L-arginine (L-arg) caused a significant decrease in SNP induced relaxations (6–55%; $p < 0.05$, $p < 0.001$ and 2–46%; $p < 0.05$, $p < 0.01$ for ES- and 15–28%; $p < 0.05$, $p < 0.01$, $p < 0.001$ and 12–32%; $p < 0.05$, $p < 0.01$ for Ach-induced contractions, respectively). In conclusion, our data suggest that SNP, which releases NO, is able to depress longitudinal muscle contraction of the isolated guinea pig ileum, suggesting that exogenous application of NO inhibits intestinal contractions of smooth muscle cells and that cGMP mediates the response to NO. In addition, MnSOD and L-arg decreased the relaxing effect of SNP on the isolated ileum of the guinea pig.

1. Introduction

Nitric oxide (NO) is the active chemical species responsible for the vasodilator action of sodium nitroprusside (SNP), nitroglycerin and related nitrovasodilators. NO itself can account for the vasodilator action of each of the nitrovasodilator drugs studied (Ignarro et al. 1981). SNP (an inorganic nitroso compound) is a potent vasodilator, releasing NO directly in a nonenzymatic fashion (Abrams et al. 1996).

NO is created from the terminal group of L-arginine (L-arg), in a reaction catalyzed by NO-synthase (NOS). NOS catalyzes the initial step of L-arg conversion to NO, which includes nitrogen hydroxylation of guanidine-group of L-arg (Palmer et al. 1988). NO or a NO-related compound are now widely recognized as important physiological mediators of nonadrenergic-noncholinergic relaxation of gastrointestinal (GI) smooth muscle (Sanders and Ward 1992). Relaxation following neuronal stimulation has been reported in a wide range of GI smooth muscles, including duodenum and ileum in rat and guinea pig (Irie et al. 1991; Kanada et al. 1992) and dog duodenum and colon (Toda and Herman 2005; Ward et al. 1992). The mechanism of NO action in the GI tract is not fully understood, but many of its actions are mediated by the activation of soluble guanylate cyclase (sGC), resulting in an increase in concentration of cyclic guanosine 3′,5′-monophosphatate (cGMP) in smooth muscle cells (Ignarro et al. 1986). cGMP is thought to act directly on ion channels and activate protein kinase, regulating the activity of proteins through their phosphorylation (Robertson et al. 1993).

NO is one of the active oxidant molecules, a group including superoxide anions (O^{2-}), hydrogen peroxide, hydroxyl radicals, peroxynitrite ($ONOO^-$) and lipid-derived radicals. Immunohistochemical studies have revealed the superoxide dismutase (SOD), which is a scavenger for O^{2-}, in neuronal NOS-containing nerves and which would have the potential of protecting NO from destruction by O^{2-} (Liu et al. 1996). O^{2-} binds rapidly with NO to form the highly reactive $ONOO^-$ (Beckman and Koppenol 1996), an intermediate that is much less potent in stimulating sGC. O^{2-} can also reduce vascular responses to exogenous and endogenous NO donors (Munzel et al. 1996).

The aims of this study were two-fold: to determine (1) whether the NO-donating compound, SNP, induces relaxation of the longitudinal muscle isolated from the guinea pig ileum and (2) whether sCG is involved in SNP-mediated relaxation of the isolated guinea pig ileum. Specifically, we studied the effects of the manganese-containing SOD (MnSOD) and L-arg on the relaxing effect of SNP in guinea pig ileum.

2. Investigations and results

All experiments were performed in Tyrode solution, in order to examine whether NO donor SNP induces relaxation of longitudinal muscle of isolated guinea pig ileum. After an equilibration period, the ileal segments were contracted by electrical stimulation (ES) (3 Hz, 60–90V, 0.3 ms, 3 s) and administration of 0.5 μM acetylcholine (Ach). After achieving stable contractions, the tissues were exposed to increasing concentrations of SNP (10^{-10}M, 10^{-9}M, 10^{-8}M, 10^{-7}M, 10^{-6}M and 10^{-5}M). Relaxant responses were expressed as percentage of control of ES- and Ach-induced contractions. The obtained results show that SNP reduced ES- by 8–76%; $p<0.05$ and Ach-stimulated contractions by 23–62%; $p<0.05$ of the guinea pig ileum significantly and in dose-dependent manner (Fig. 1).

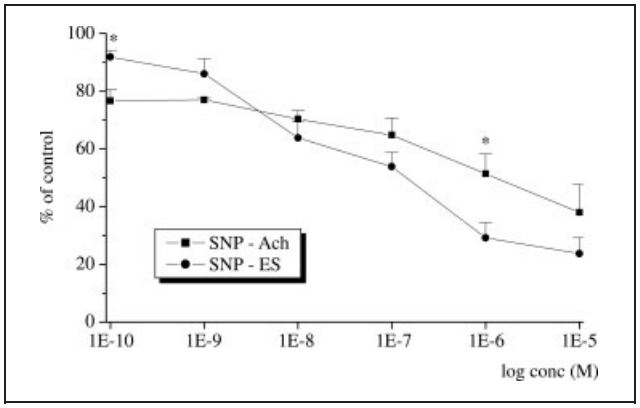

Fig. 1: Dose-dependent effects of SNP on ileum contraction.
Depressant action of graded concentrations of SNP (10^{-10}M, 10^{-9}M, 10^{-8}M, 10^{-7}M, 10^{-6}M and 10^{-5}M) on isolated guinea pig ileum contractions induced by ES (3 Hz, 60–90 V, 0.3 ms, 3 s) and 0.5 μM Ach (1.5 min). Values of control were arbitrarily assigned as a 100% and values for the treatment were expressed as a % of control. Each data point is the mean ±SEM of 4 to 6 experiments.
SNP indicates sodium nitroprusside; ES indicates electrical stimulation; Ach indicates acetylcholine; * $p < 0.05$

In order to examine whether the relaxing effects of SNP were due to activation of sGC, we then performed similar experiments, in the presence of the sGC inhibitor, methylene blue (MB) in the medium. Figure 2 presents group of data including the effects of MB (10 mM), continuously present in Tyrode solution) on ES- and Ach-stimulated contraction in the presence of increasing concentrations of SNP. It can be observed that MB reduced SNP-stimulated relaxation significantly, by 0–39%; $p < 0.05$, $p < 0.01$, $p < 0.001$ for ES- (A) and by 4–27%; $p < 0.05$ for Ach-induced contractions (B).

To examine whether MnSOD, a scavenger of superoxide anions, affects SNP-induced relaxation, the SNP-stimulated relaxations were measured following treatment with the 1 μM MnSOD for 1 min. It is apparent that MnSOD presence significantly antagonizes the relaxing effect of SNP on contraction induced by ES by 6–55%; $p < 0.05$,

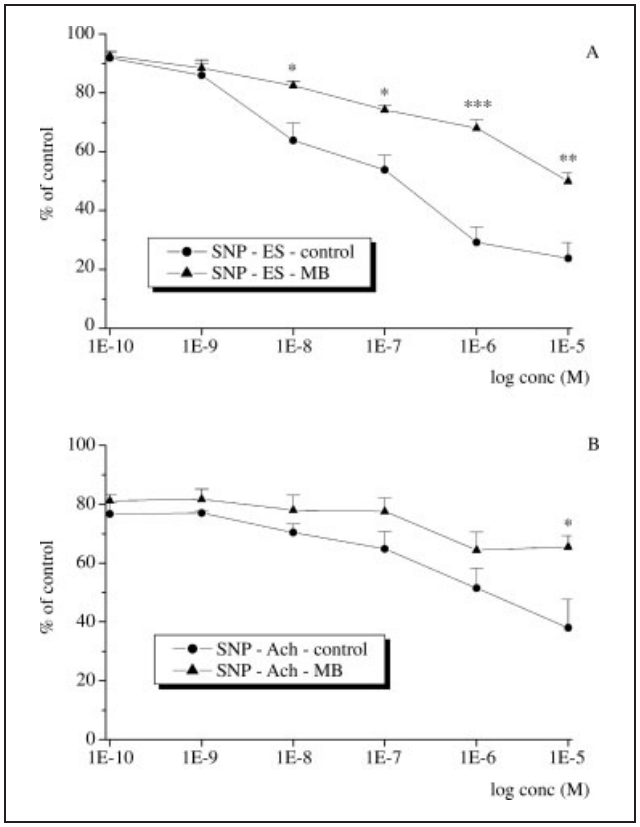

Fig. 2: Effect of MB on ileum contractions in the presence of SNP.
Depressant action of graded concentrations of SNP (10^{-10}M, 10^{-9}M, 10^{-8}M, 10^{-7}M, 10^{-6}M and 10^{-5}M) on isolated guinea pig ileum contractions induced by ES (A) and Ach (B) in the presence of MB (10 mM, continuously presented in Tyrode solution). Values of control were arbitrarily assigned as a 100% and values for the treatment were expressed as a % of control. Each data point is the mean ±SEM of 4 or 5 experiments.
MB indicates methylene blue; ** $p < 0.01$; *** $p < 0.001$. Other abbreviations as for Fig. 1

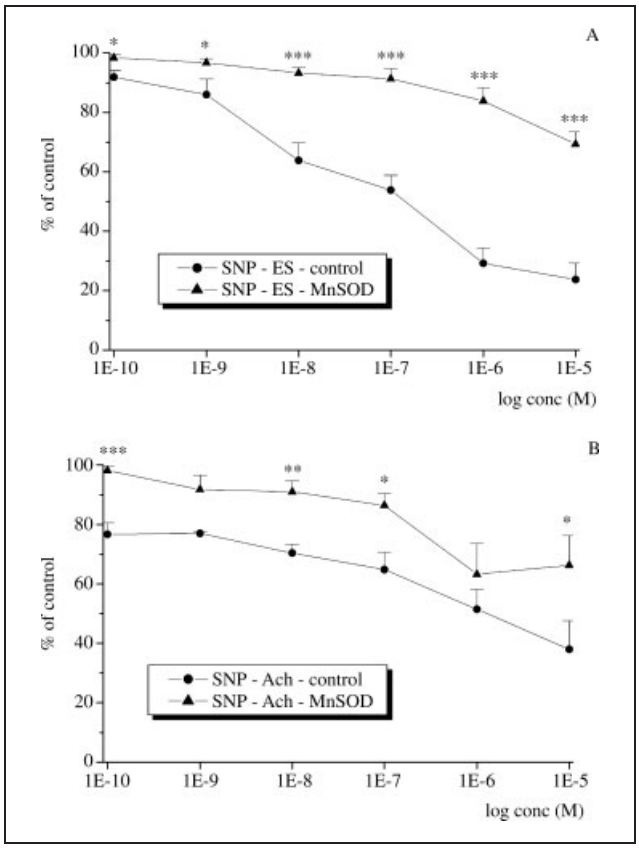

Fig. 3: Effects of MnSOD on SNP-induced relaxation.
Depressant action of graded concentrations of SNP (10^{-10}M, 10^{-9}M, 10^{-8}M, 10^{-7}M, 10^{-6}M and 10^{-5}M) on isolated guinea pig ileum contractions induced by ES (A) and Ach (B) in the presence of MnSOD (1 μM, 1min). Values of control were arbitrarily assigned as a 100% and values for the treatment were expressed as a % of control. Each data point is the mean ±SEM of 4 to 7 experiments.
MnSOD indicates manganese-containing superoxide dismutase; * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Other abbreviations as for Fig. 1

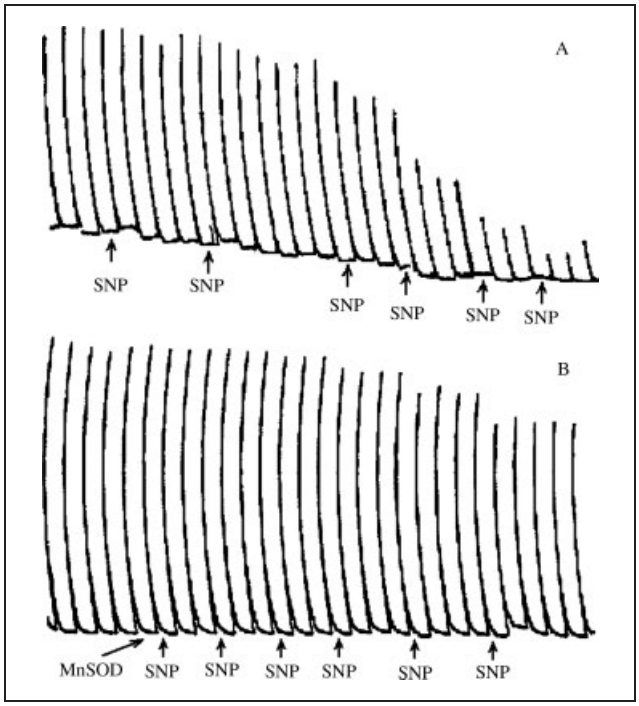

Fig. 4: A representative trace of the response of isolated guinea pig ileum contractions induced by ES treated with increasing concentrations of SNP (10^{-10}M, 10^{-9}M, 10^{-8}M, 10^{-7}M, 10^{-6}M and 10^{-5}M) in the absence of (A) and in the presence of MnSOD (1μM) (B) in the medium. MnSOD indicates manganese-containing superoxide dismutase. Other abbreviations as for Fig. 1

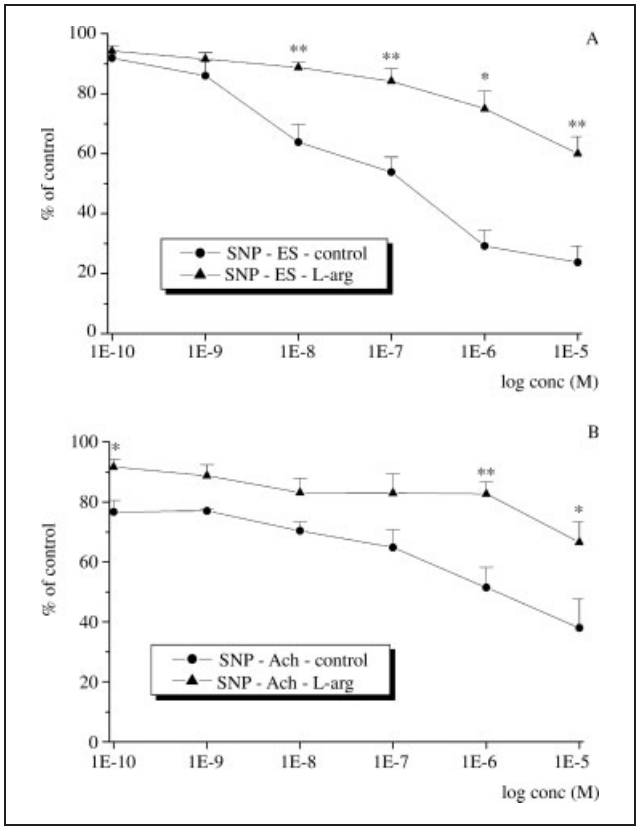

Fig. 5: Effects of L-arg on SNP-induced ileum contractions.
Depressant action of graded concentrations of SNP (10^{-10}M, 10^{-9}M, 10^{-8}M, 10^{-7}M, 10^{-6}M and 10^{-5}M) on isolated guinea pig ileum contractions induced by ES (A) and Ach (B) in the presence of 10 μM L-arg added 3 min before adding SNP. Values of control were arbitrarily assigned as a 100% and values for the treatment were expressed as a % of control. Each data point is the mean ±SEM of 3 to 5 experiments.
L-arg indicates L-arginine $^{*}p < 0.05$; $^{**}p < 0.01$. Other abbreviations as for Fig. 1

$p < 0.001$ (Fig. 3A and Fig. 4) and by Ach by 15–28%; $p < 0.05$, $p < 0.01$, $p < 0.001$ (Fig. 3B). On its own, MnSOD did not affect the ES- or Ach-induced contractions.

Figure 5 shows the effect of L-arg on SNP-stimulated relaxation. Increasing concentrations of SNP were added to isolated guinea pig ileum which was previously contracted by ES and by Ach in the presence of L-arg (10 μM) in the medium. L-arg was added to the medium 3 min before adding SNP. It can be seen that L-arg has a significant inhibitory effect on SNP-stimulated relaxation (2–46%; $p < 0.05$, $p < 0.01$ for ES- (A) and 12–32%; $p < 0.05$, $p < 0.01$ for Ach-induced contractions (B)).

3. Discussion

In light of the evident importance of NO in intestinal physiology and pathology, in this study we have explored the effects of the NO-donating compound SNP on the longitudinal muscle relaxation of the isolated guinea pig ileum. To test whether cGMP mediates the response to NO, we used MB, which is known to inhibit sGC. In addition, the aim of these studies was to examine the influence of MnSOD and L-arg on the relaxing effect of SNP on the isolated ileum of the guinea pig.

The results of this study show that SNP is able to depress, in a concentration-dependent manner, the contractions of the isolated guinea pig ileum induced by ES and by Ach (Fig. 1), indicating that exogenous application of NO inhibits intestinal contractions of smooth muscle cells. The effect of the nitrergic transmitter released from enteric nerves shows similarities with the effects caused by SNP (Tanovic et al. 2001). It has been demonstrated that NO is able to activate sGC. At the same time, MB has been used by many investigators to determine whether cGMP is involved in the transduction of agonist effects, because it can inhibit sGC (Lefebvre and Bartho 1997; Moncada and Higgs 1993; Izzo et al. 1996). In our experiments, MB was able to reverse SNP-induced relaxation (Fig. 2). Our results indicate that NO-induced relaxations in guinea pig ileum are related to the activation of sGC and an increase in cGMP level. The concentration of MB required to reduce the NO-induced relaxation in the small intestine was higher than in vascular preparations (Gruetter et al. 1981; Martin et al. 1985). A similar moderate inhibitory effect of MB on nitrergic relaxations has been observed in other GI preparations (Huizinga et al. 1992; Lefebvre et al. 1995).

SNP catabolises to NO^+ (nitrosonium) redox form that interacts with sulphydril groups of available proteins and makes S-nitrosothiols (SNO). SNO yields NO which stimulates sGC and thus increases concentration of cGMP causing vasorelaxation. During this catabolism, 70% of SNP yields NO^+ redox form, while only 30% yields NO radical (Orescanin and Milovanovic 2006). Data acquired from the current research provide evidence that the presence of MnSOD in the medium decreases SNP-mediated relaxation of the isolated guinea pig ileum (Fig. 3 and Fig. 4). These results are in accordance with our previously published results on isolated rat renal artery (Milovanovic et al. 2004) which suggests that MnSOD converts NO and reduces cGMP dependent relaxation. The generation of NO^+ and nitroxyl ions (NO^-/HNO) by NO-

treated MnSOD, which produces both enzyme modifications and inactivation, has been demonstrated (Niketic et al. 1999; Filipovic et al. 2007). Exposure of MnSOD to NO led to its fast and extensive inactivation, which was accompanied by extensive structural alterations, including the cleavage of enzyme polypeptide chains, presumably at histidine residues of the enzyme metal binding sites (Niketic et al. 1999). We have previously found, as other have, that the differential chemical behavior of NO and HNO toward haem proteins offers a unique control mechanism for the biological action of NO (Moncada et al. 1991; Milovanovic et al. 2004). Our results showed that MnSOD inhibits the relaxation effect of SNP in the examined ileum, by modifying the chemical versatility of NO into redox active forms, NO^+ and NO^-, producing relaxation effects in the guinea pig ileum which are in accordance with our previously published results (Milovanovic et al. 2004).

Another finding that was discovered during this research was that L-arg, like MnSOD, antagonizes the relaxing effect of SNP in the examined ileum of the guinea pig significantly (Fig. 5), regardless of the fact that L-arg produces vascular relaxation by itself (McCall and Vallance 1992; Prostran et al. 1994). Our previous results (Orescanin and Milovanovic 2006) also show that L-arg partly antagonized relaxation evoked by SNP on isolated rat renal artery, producing directly NO-radical, that occupies the whole sGC and, in current study, prevents effects of SNP. L-arg as well as MnSOD, produces smaller relaxing effects of SNP in guinea pig ileum by modifying chemical versatility of NO into redox active forms NO^+ and NO^-.

In conclusion, our data suggest that L-arg and MnSOD affect the relaxation of the isolated ileum of the guinea pig induced by of SNP and that cGMP mediates the response to NO. Since NO is involved in the intestinal physiology and pathology and the subsequent formation of many important secondary messengers, an understanding of its role in the regulation of relaxation and contractility in the gut may provide some insight into the role of NO in intestinal pathophysiology.

4. Experimental

4.1. Methods

Healthy guinea pigs of both sex (3 months old; 250–300 g b. wt.) were maintained at 22 °C with 12/12 h light-dark schedule (free access to food and water). The animal were used after an overnight fasting and sacrificed by decapitation and bleeding *via* carotid arteries. The terminal ileum was rapidly removed and suspended in Tyrode buffer (containing in mM: NaCl, 137; KCl, 2.7; NaH_2PO_4, 0.4; $MgCl_2$, 1.0; $CaCl_2$, 1.8; $NaHCO_3$, 12.0 and (+)-glucose, pH 5.5). The tissue was carefully cleaned and the contents in the excised segments were gently flushed out with Tyrode solution. Tissue strips (15–20 mm wide), were suspended to the tissue holder and placed between two platinum plates, located in 40-ml baths. Segments were washed twice and equilibrated with a resting tension of 1 g over a 30-min period with rinsing each 10 min. The Tyrode solution was aerated continuously with $95\%O_2/5\%CO_2$ and maintained at 37 °C. The upper end of the strip was connected to the isometric force transducer (Ugo Basile, 21025 Comerio, Italy) with the force displayed on a recording microdinamometer 7050 (Ugo Basile), to register mechanical contraction. Two platinum plates were connected to the electrical stimulator (IPM Elektronik-Tuzla, Bosnia and Herzegovina). The sensitivity of the recording instrument was kept at 4.0 throughout the experiments. The paper speed was 2 mm/min.

After an equilibration period, the ileal segments were contracted by ES (3 Hz, 60–90 V, 0.3 ms, 3 s) or by administration of 0.5 μM Ach for 1.5 min. Tissues were washed and allowed to equilibrate for additional 5 min. After equilibration, increasing concetrations of SNP (10^{-10}M, 10^{-9}M, 10^{-8}M, 10^{-7}M, 10^{-6}M and 10^{-5} M) were added to isolated guinea pig ileum for 3min. The same procedure was repeated in the presence of 10 mM MB (continuously presented in Tyrode buffer), 1 μM MnSOD (1 min) or 10 μM L-arg (3 min) in the medium. The effects of all compounds were investigated either by adding them up in a cumulative fashion to construct a concentration–response curve (ES-type contraction) or at a single concentration (Ach-type contraction). The results were expressed as a percentage of the mean of at least three predrug ES-induced responses, which was expressed as a 100%. The effect of the compounds in a single strip was determined from at least two Ach-induced contractions.

4.2. Drugs and solutions

In the present study, the following drugs were used: SNP obtained from Kemika (Zagreb, Croatia) and L-arg purchased from Merck (USA), Ach purchased from Serva-Feinbiochemica (Germany). MnSOD was isolated according to Keele et al. (1970).

All drugs were dissolved in distilled water and stored at 4 °C. SNP was protected from exposure to light. Drugs were added into the organ bath in volumes less than 1% of the bathing solution (this volume did not affect the spontaneous contractile activity or muscle tone).

4.3. Statistical analysis

Results are expressed as mean ±S.E.; n represents the number of separate experiments. Mechanical activity is expressed as a percentage of the control values (100%). Statistical differences between paired experiments were assessed by Students's test. P values less than 0.05 were considered significant (compared with control unless otherwise specified).

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