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# Biphasic effects of NMDA on the motility of the rat portal vein

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**1** The effect of NMDA on the motility of the rat portal vein was studied in an isolated preparation. NMDA induced a concentration-dependent ($10^{-7}$–$10^{-4}$ M) increase of the contraction frequency (maximum increase, $148 \pm 6\%$ of control at NMDA $10^{-4}$ M).

**2** The NMDA-induced excitatory response was prevented by the competitive NMDA receptor antagonists (±)-2-Amino-5-phosphonopentanoic acid (AP-5, $5 \times 10^{-4}$ M) or (RS)-3-(2-carboxypiperazine-4-yl) propyl-1-phosphonic acid (CPP, $10^{-4}$ M).

**3** Tetrodotoxin (TTX, $10^{-6}$ M) or atropine ($10^{-4}$ M) abolished the NMDA-induced increase of the portal vein motility and reversed the excitatory effect to a concentration-dependent inhibition (maximum inhibition, $52 \pm 8$ and $29 \pm 7\%$ of controls, respectively, at NMDA $10^{-3}$ M).

**4** Removal of the endothelium abolished the NMDA-induced inhibitory response. Sodium nitroprusside concentration-dependently ($10^{-7}$–$10^{-5}$ M) inhibited the portal vein motility, while L-$N^G$-nitro-arginine methyl ester (L-NAME, $10^{-4}$ M) reversed the inhibitory effect of NMDA (in the presence of TTX), restoring the portal vein spontaneous activity to control values.

**5** These results show that NMDA modulates the portal vein motility in a biphasic manner: *via* indirect activation, through prejunctional NMDA receptors presumably located on intrinsic excitatory neuronal afferences, or *via* direct inhibition, through endothelial NMDA receptors activating the nitric oxide pathway. Overall these findings support the hypothesis of the existence of a peripheral glutamatergic innervation modulating the contractile activity of the rat portal vein.
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**Keywords:** Nitric oxide; NMDA receptors; atropine; vascular smooth muscle; peripheral glutamatergic system

**Abbreviations:** (±)-AP-5, (±)-2-Amino-5-phosphonopentanoic acid; CPP, (RS)-3-(2-carboxypiperazine-4-yl) propyl-1-phosphonic acid; L-NAME, L-$N^G$-nitro-arginine methyl ester; NOS, nitric oxide synthase; TTX, tetrodotoxin

## Introduction

Increasing evidence suggests that glutamate, the major excitatory neurotransmitter in the CNS (Fonnum, 1984), may also be a neurotransmitter in the peripheral nervous system. Excitatory effects of glutamate on peripheral tissues have long been described (Nistri *et al*., 1979; Moroni *et al*., 1986). Binding studies have demonstrated the presence of NMDA glutamate receptors in the myenteric plexus (Moroni *et al*., 1986), ileal longitudinal muscle (Luzzi *et al*., 1988), bronchi (Aas *et al*., 1989), adrenal glands (Yoneda *et al*., 1986), cardiocytes (Winter & Baker, 1995). There is also physiological and electrophysiological evidence for the existence of AMPA and kainate subtype of glutamate receptors in pancreatic islets (Bertrand *et al*., 1992; 1993; 1995; Weaver *et al*., 1996). More recent biochemical and neuroanatomical studies have shown the presence of glutamatergic neurones as well as mRNA for the subunit of the NMDA glutamate receptor NR1 in the enteric nervous system (Wiley *et al*., 1991; Liu *et al*., 1997) where glutamate-mediated neurotransmission appears to be mediated primarily by NMDA and AMPA subtypes of glutamate receptors. The expression of the NR1 subunit has also been documented in human keratinocytes and cardiocytes (Morhenn *et al*., 1994) while mRNA encoding for the AMPA subunits receptor were found in the rat adrenal gland (Kristensen *et al*., 1993). Taken together, these studies indicate that NMDA glutamate receptors are present on nerve inputs or on contractile cells themselves, or both, in a variety of contractile peripheral tissues and suggest that the motility of these tissues might be physiologically modulated by a peripheral excitatory glutamatergic transmission. It is not known, however, whether glutamate receptors are present in vascular tissues or whether the contractility of the vascular bed can be modulated by NMDA.

The longitudinal muscle of the rat portal vein exhibits spontaneous intermittent rhythmic contractions resembling in many respects the vasomotor activity observed in other parts of the circulatory system. The isolated preparation of the portal vein is a multicellular preparation activated by myogenic spread of excitation and represents a well standardized model for studying the cellular mechanism by which vasoactive agents influence the contractile activity of the blood vessels.

Thus, we used this *in vitro* preparation for studying the effects of NMDA on the spontaneous rhythmic activity in order to assess whether glutamate NMDA receptors might be involved in the regulation of the contractile activity of the rat portal vein.

## Methods

### *Animals*

Twenty male Wistar rats (Charles River) weighing 250–300 g were used. The animals were kept six per cage for 1 week before the onset of the experiments. The rats were maintained under constant photoperiod conditions (12 h dark, 12 h light) at a temperature of 23°C and a relative humidity of 60%. Standard pelleted laboratory rat food and water were available *ad libitum*.

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### Tissue preparation

During deep ether anaesthesia, the abdomen was opened by a parasagittal incision. Under stereo microscope (40×), the portal vein (5 mm *in situ* length) was carefully dissected from the surrounding tissue and then cut both at the level of the gastrosplenic vein and its bifurcation at the liver hilum. The vessel was then immediately placed into modified (magnesium free) Krebs buffer pH 7.4, with a composition (mM): NaCl, 118; KCl, 4.70; $CaCl_2$, 2.52; $NaHCO_3$, 24.88; Glucose, 5.55.

### Experimental studies of vascular segment

Portal veins were mounted in a 10 ml organ bath through which Krebs solution, (bubbled with 95% $O_2$ and 5% $CO_2$), flowed continuously (2.5 ml $min^{-1}$) at a temperature of 37°C *via* a thermostatically controlled perfusion pump (Basile, Italy). With one end of the portal vein firmly fixed to the bottom of the bath, the tension along the longitudinal axis of vascular segment was recorded by attaching the other end to a MLT100 (McLab) isometric force transducer. The transducer was mounted on a moving support allowing a minimal length increment of 5 $\mu$m. It was then adjusted manually until the first increase in tension was recorded in the following manner. During an equilibration period of 1 h, the portal vein was gradually stretched until it reached its *in vivo* length. This length was measured with a microscope and established as the base line length, indicated as $L_o$. At $L_o$ spontaneous contractile activity appeared. An accommodation period (20–50 min) during which spontaneous mechanical activity became stable, was allowed. The portal veins were elongated in increments of 200 $\mu$m (5% of the *in vivo* length, $L_o$), until the optimum length for maximal amplitude of contraction waves was reached.

In some experiments, the motility of the portal vein was recorded after mechanical removal of the endothelium by gently rubbing the intimal surface of the vessel with a small cotton ball.

### Drugs

N-methyl-D-aspartate (NMDA), inhibitor L-$N^G$-nitro-arginine methyl ester (L-NAME), atropine, tetrodotoxin (TTX) and (±)-2-Amino-5-phosphonopentanoic acid ((±)-AP-5) were obtained from Sigma (St Louis, MO, U.S.A.), (RS)-3-(2-Carboxypiperzin-4-yl)-propyl-1-phosphonoc acid (CCP) was from Tocris (Langford, Bristol, U.K.). Sodium nitroprusside was from Farmitalia Carlo Erba Reactifs (Milano, Italy).

### Data analysis

Each spontaneous force wave was measured in amplitude during 5 min control period and a subsequent 5 min after application of each cumulative dose. The frequency of contraction waves was evaluated by computing the number of contractile events in a period of 5 min and reported as cycles $min^{-1}$.

### Statistics

The data were analysed with a one-way ANOVA, and *post hoc* comparisons were conducted using the Newman-Keuls test or Dunnett's Multiple Comparisons test when necessary. Points are presented as mean±s.e.mean of normalized values (per cent of baseline values).

## Results

The spontaneous activity of the longitudinal smooth muscle of the portal vein in standard Krebs solution was characterized by a pattern of uniform phasic contractions which occurred with regular frequency (4±0.3 waves $min^{-1}$, mean±s.e.mean, $n=287$) and amplitude (0.06±0.003 newtons, mean±s.e.mean). Recordings of this activity are illustrated in the pre-drug period of Figure 1A.

### Effect of NMDA on the contractile activity

The addition to the bath of cumulative doses of NMDA ($10^{-7}$–$10^{-4}$ M) induced a concentration-dependent increase of the contraction frequency (Figure 1A) which reached a maximum of 148±6% at NMDA $10^{-4}$ M ($F_{(5, 109)}=9.15$, $P>0.001$) if compared to pre-drug values (Figure 1B). Elevating the NMDA concentration to $10^{-3}$ M produced no further increase in the contraction frequency. At doses of $10^{-7}$–$10^{-3}$ M, NMDA had no significant effect on the amplitude of contraction waves ($F_{(5, 108)}=2.21$, $P=0.059$; Figure 1B). The motility index of NMDA-induced response, calculated as the product of the amplitude by the frequency of contraction waves, indicated a significant increase of portal vein motility (maximum increase: 159±7% of baseline at NMDA $10^{-4}$ M; $F_{(5, 99)}=6.213$, $P<0.0001$; Figure 1C).

### The competitive NMDA-receptor antagonist (±)-AP-5 or CPP prevents the NMDA-induced excitatory response

To assess whether these effects were mediated by NMDA receptors we measured the NMDA-induced contractile response of the portal vein in the presence of the NMDA receptor antagonists (±)-AP-5 or CCP. The addition of (±)-AP-5 alone ($5\times10^{-4}$ M) did not produce a significant change of the frequency ($P=0.20$, unpaired *t*-test). However, (±)-AP-5 prevented the NMDA-evoked increase of the contraction frequency ($F_{(9, 111)}=6.7$, $P<0.0001$; Figure 2A). The motility index showed a significant decrease of the overall portal vein motility (maximum decrease: 74±4% of baseline at NMDA $10^{-4}$ M; $F_{(9, 111)}=9$, $P<0.0001$; Figure 2B). The addition of another NMDA receptor antagonist, CPP, produced similar results. CPP was ineffective when added alone ($P=0.12$, unpaired *t*-test), but prevented the NMDA-induced increase in the portal vein contractile response ($F_{(9, 109)}=5.89$, $P<0.0001$; Figure 2B).

### Effect of TTX on the excitatory response induced by NMDA

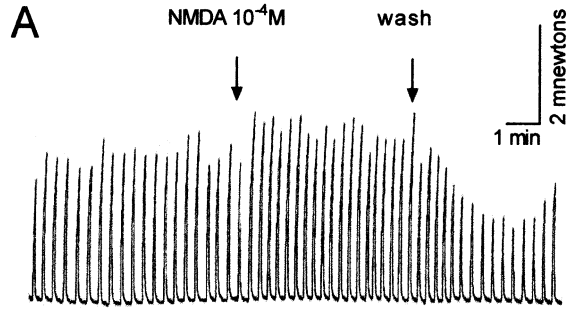
In order to verify whether the effects of NMDA were mediated indirectly through intrinsic nerve afferences, the NMDA-induced response was studied in the presence of the $Na^+$ channel blocker TTX. TTX ($10^{-6}$ M) had no effect on the frequency or amplitude of contraction waves. However, TTX completely inhibited the NMDA-induced increase in frequency ($F_{(9, 104)}=5.762$, $P<0.0001$; Figure 3A). Surprisingly, in the presence of TTX, NMDA ($10^{-7}$–$10^{-3}$ M) induced a concentration-dependent decrease in the wave amplitude (maximum decrease: 52±4% of baseline at NMDA $10^{-3}$ M, $F_{(9, 103)}=9.913$, $P<0.0001$; Figure 3B). The motility index of the NMDA response in the presence of TTX was significantly reduced (maximum decrease, 48±8% of baseline at NMDA $10^{-3}$ M, $F_{(9, 104)}=15.12$, $P<0.0001$; Figure 3C).

*Atropine reduces the NMDA-induced excitatory response*

To evaluate the involvement of intramural cholinergic neurotransmission in the excitatory effects of NMDA, we studied the action of atropine on the NMDA-induced response. Atropine alone ($10^{-6}$ M) had no effect on the spontaneous activity of the portal vein ($P>0.05$, unpaired $t$-test). However atropine prevented the excitatory effects of NMDA ($10^{-7}-10^{-3}$ M) on the contraction frequency (Figure 4A). Nevertheless, in the presence of atropine, NMDA ($10^{-3}$ M) induced a significant inhibition of the frequency of contraction waves (maximum inhibition: $80\pm8\%$ of baseline; $F_{(9,\ 105)}=5.6$, $P<0.0001$). The motility index of the NMDA response in the presence of atropine also was significantly reduced (maximum reduction, $71\pm8\%$ of baseline at NMDA $10^{-3}$ M, $F_{(9,\ 105)}=5.72$, $P<0.0001$; Figure 4B).

*Removal of the endothelium abolishes the NMDA-induced inhibition of contractile activity*

To examine further the mechanism of the inhibition by NMDA in the presence of nerve impulse blockade by TTX, we first assessed whether the endothelium might contribute to the inhibitory response. As shown in Figure 5, the inhibitory effect of NMDA ($10^{-4}$ M), was completely abolished after removal of the endothelium ($F_{(3,\ 47)}=9.0$, $P<0.0001$).



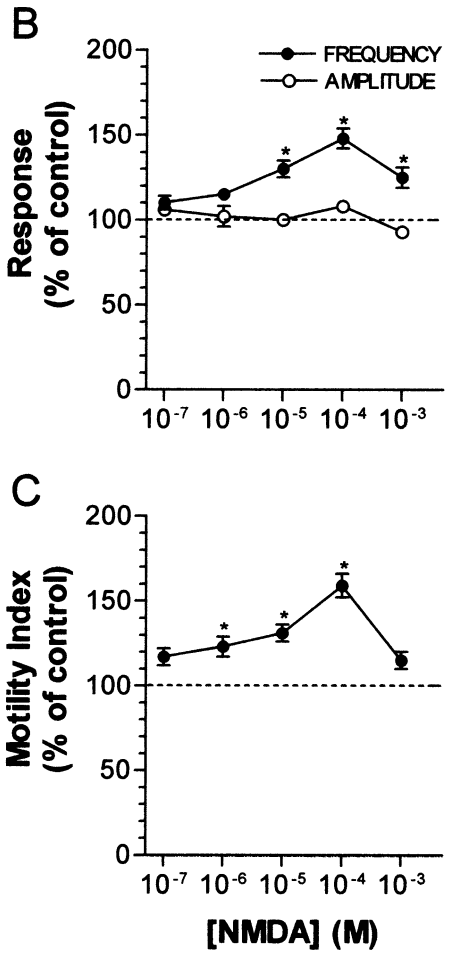




**Figure 1** (A) Representative experimental traces showing the effect of NMDA ($10^{-4}$ M) on the contraction waves in the isolated rat portal vein preparation. (B) Concentration-response curve of the frequency and amplitude of portal vein contraction activity. The points are presented as per cent of baseline values of the spontaneous activity obtained before the exposure to NMDA. Each point is the mean$\pm$s.e.mean from four independent experiments. NMDA produced a significant main effect on the frequency ($F_{(5,\ 109)}=9.15$, $P<0.0001$; one-way ANOVA). (C) Motility index for the NMDA response. NMDA produced a significant main effect on the motility on the portal vein ($F_{(5,\ 99)}=6.213$, $P<0.0001$). Values represent per cent of the baseline motility. *$P<0.05$ with respect to baseline values (Newman-Keul's test).

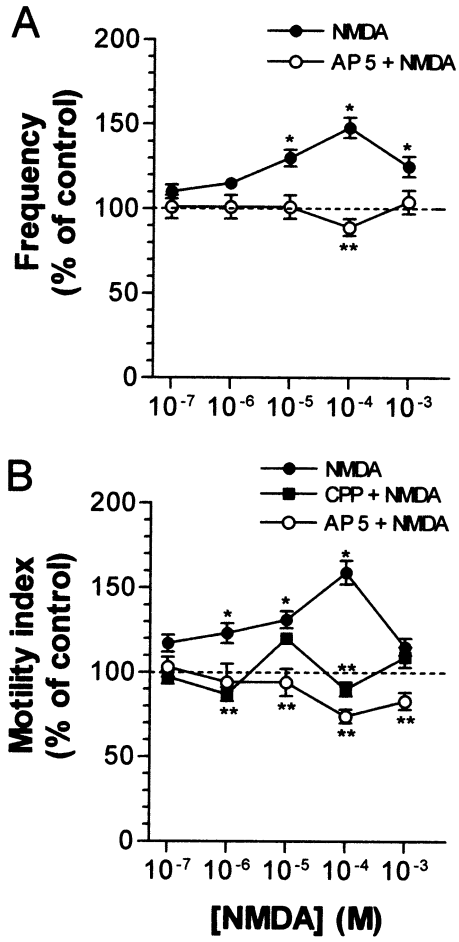



**Figure 2** Effect of the competitive NMDA receptor antagonist ($\pm$)-AP-5 on NMDA-induced increase of the frequency of the contraction waves of the rat portal vein. ($\pm$)-AP-5 ($5\times10^{-4}$ M) alone had no significant effect on the frequency of contraction waves ($P=0.20$, unpaired Student's $t$-test; data not shown). (A) ($\pm$)-AP-5, added 5 min before NMDA, abolished the NMDA-induced increase of contraction frequency ($F_{(9,\ 111)}=6.7$, $P<0.0001$). (B) ($\pm$)-AP-5 or CPP significantly prevent the NMDA-induced increase of motility of the rat portal vein ($F_{(9,\ 105)}=5.72$, $P<0.0001$ and $F_{(9,\ 109)}=5.89$, $P<0.0001$ respectively). Points are shown as per cent of basal values of the spontaneous contraction activity measured before the exposure to the drug and are the mean$\pm$s.e.mean from two independent experiments. *$P<0.05$ with respect to baseline values; **$P<0.05$ as compared to NMDA (Newman-Keul's test).

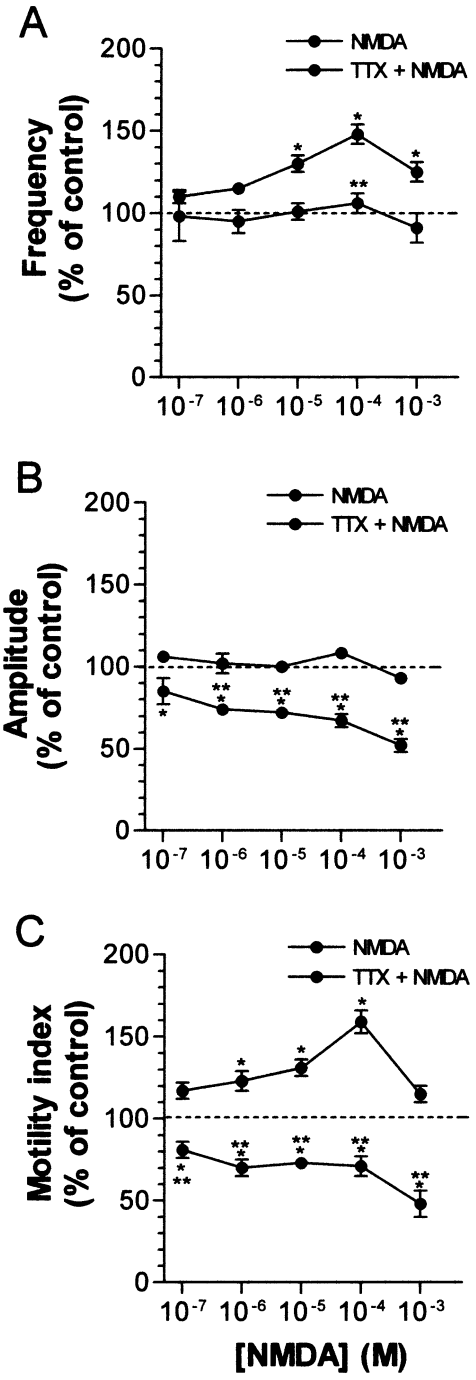



**Figure 3** Effect of TTX ($10^{-6}$ M) on the NMDA-induced excitatory effect on the portal vein contraction activity. (A) TTX abolished the NMDA-elicited increase in the contraction frequency ($F_{(9, 104)}=5.76$, $P<0.0001$). (B) In the presence of TTX, NMDA produced a reduction in the contraction amplitude ($F_{(9, 103)}=9.91$, $P<0.0001$) and (C) in the motility index ($F_{(9, 104)}=15.12$, $P<0.0001$). Points are presented as per cent of baseline values of the spontaneous contraction activity obtained before the exposure to the TTX and are the mean ± s.e.mean from two independent experiments. $^{*}P<0.05$ with respect to baseline values; $^{**}P<0.05$ as compared to NMDA (Newman-Keul's test).

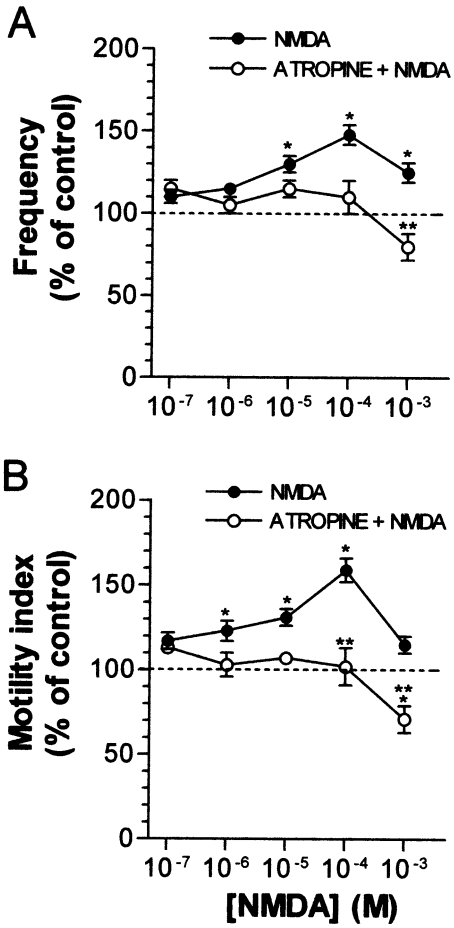



**Figure 4** Effect of atropine ($10^{-6}$ M) on the NMDA-induced increase of the rat portal vein contraction activity. (A) Atropine blocked the NMDA-evoked increase in the frequency of contraction waves ($F_{(9, 105)}=5.6$, $P<0.0001$). (B) The motility was significantly reduced in the presence of atropine ($F_{(9, 105)}=5.72$, $P<0.0001$). Points are presented as per cent of baseline values of the spontaneous contraction activity obtained before the exposure to the drug and are the mean ± s.e.mean from two independent experiments. $^{*}P<0.05$ with respect to baseline values; $^{**}P<0.05$ as compared to NMDA (Newman-Keul's test).

### *Effect of sodium nitroprusside on the portal vein contractile activity and reversal by L-NAME of the NMDA-induced inhibitory response*

To confirm that endothelial nitric oxide production is involved in the control of the contractile activity of the portal vein, we first studied the effect on the motility of the NO donor sodium nitroprusside. The addition of sodium nitroprusside ($10^{-7}$–$10^{-5}$ M) induced a concentration-dependent inhibition ($F_{(3, 16)}=47.09$, $P<0.0001$ Figure 6). On the other hand, the NO synthase inhibitor L-NAME ($10^{-4}$ M) potentiated the NMDA ($10^{-4}$ M)-induced excitation of the portal vein contractile activity (227 ± 14% of baseline; Figure 7A). Moreover, after blockade of the intrinsic nerve activity by TTX ($10^{-6}$ M), the addition of L-NAME ($10^{-4}$ M) completely reversed the NMDA ($10^{-4}$ M)-induced inhibition and restored the values of contractile activity to those of controls (Figure 7B). L-NAME added alone had little or no effect on the motility of the portal vein ($P>0.05$, unpaired $t$-test; Figure 7A).

## Discussion

Glutamate is the most important excitatory neurotransmitter in the central nervous system (Fonnum, 1984). Numerous recent studies have suggested that glutamatergic neurotransmission could modulate the intrinsic activity of a number of different peripheral excitable tissues in animals and humans (see Introduction). The present study demonstrates that also the spontaneous contractile activity of the portal vein can be

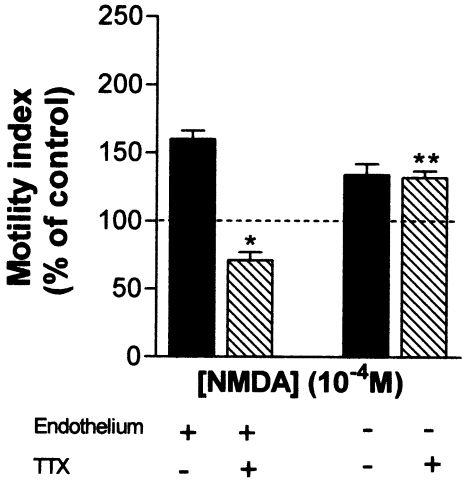



**Figure 5** Effect of the removal of the endothelium on the NMDA-induced inhibitory response. Bars represent the motility index of the NMDA-induced response ($10^{-4}$ M) in the absence or in the presence of TTX ($10^{-6}$ M), respectively. The inhibitory effect of NMDA was completely abolished after removal of endothelium ($F_{(3, 47)}=9.0$, $P<0.0001$). Values are presented as per cent of control baseline motility measured before the exposure to the drugs and are the mean±s.e.mean from two independent experiments. *$P<0.05$ as compared to NMDA-treated group **$P<0.05$ as compared to respective control group.

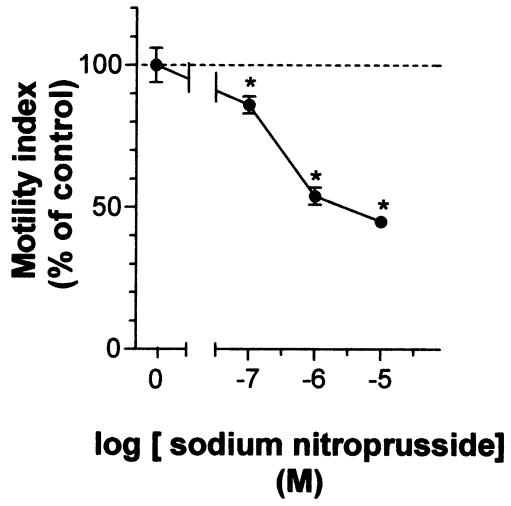



**Figure 6** Effect of the NO-donor sodium nitroprusside on the spontaneous contractile activity of the rat portal vein. Sodium nitroprusside ($10^{-7}-10^{-5}$ M) significantly reduced the motility of the rat portal vein ($F_{(3, f16)}=47.09$, $P<0.0001$). Points are shown as per cent of basal values of the spontaneous contraction activity measured before the exposure to the drug and are the mean±s.e.mean from two independent experiments. *$P<0.05$ with respect to baseline values (Dunnett's Multiple Comparisons Test).

modulated by glutamate NMDA receptors. Our results show that NMDA induces biphasic effects on the vasomotor activity of the portal vein: (i) a concentration-dependent excitation, and (ii) a concentration-dependent inhibition, which is revealed after blockade of nerve conduction by TTX. The excitatory effect on the contractile activity appears to be due to the increase in the frequency rather than the amplitude of the contraction waves. The NMDA-induced excitation was prevented by the competitive NMDA receptor antagonists (±)-AP-5 or CPP, evidence that the observed increase in contractile activity is mediated by NMDA receptors. However,

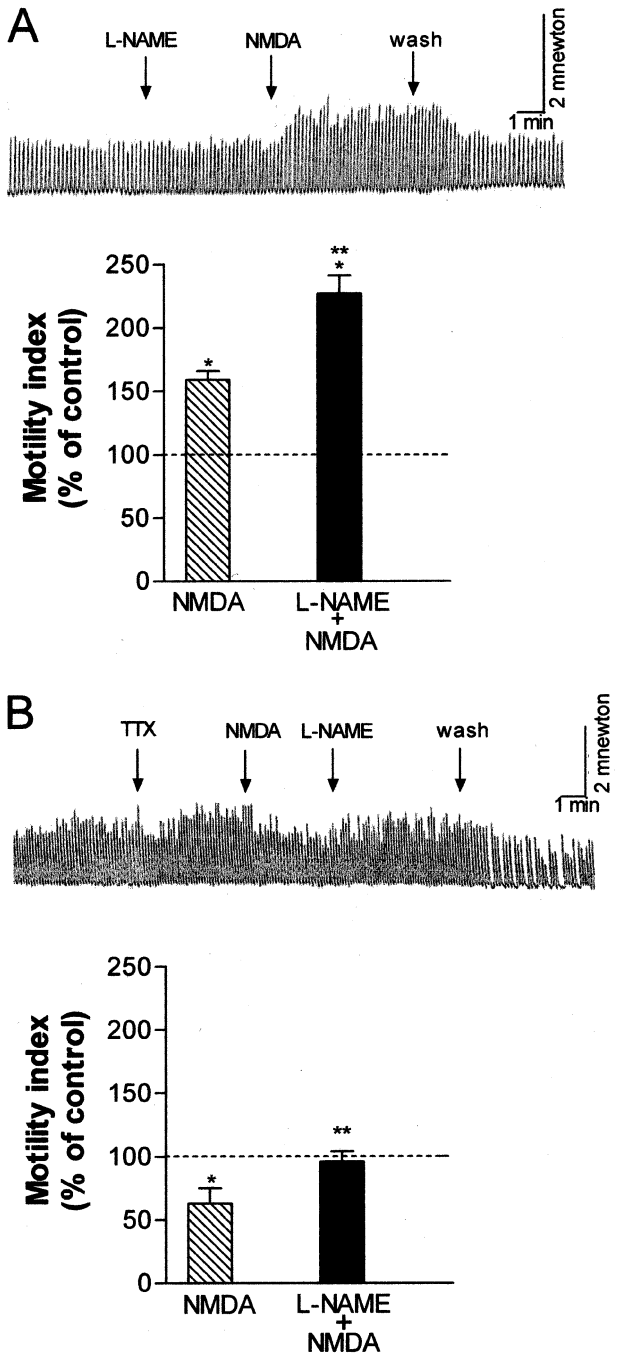



**Figure 7** (A) Representative experimental traces (top) and motility index (bottom) showing the NMDA ($10^{-4}$ M)-induced increase of the isolated rat portal vein motility in the presence of L-NAME ($10^{-4}$ M). Bars represent the motility index of the NMDA-induced effect in the absence or in the presence of L-NAME. Values are represented as per cent of control values determined before the exposure to the drugs and are the mean±s.e.mean from two independent experiments. *$P<0.05$ as compared to control values; **$P<0.05$ as compared to NMDA (unpaired Student's *t*-test). (B) Representative experimental traces (top) and motility index (bottom) showing the reversal by L-NAME ($10^{-4}$ M) of the NMDA ($10^{-4}$ M)-induced decrease in the contraction activity in the presence of TTX ($10^{-6}$ M). Bars represent the motility index of the NMDA response in the presence of TTX before and after the addition of L-NAME. Values are presented as per cent of control baseline motility measured before the exposure to the drugs and are the mean±s.e.mean from two independent experiments. *$P<0.05$ as compared to control values; **$P<0.05$ as compared to NMDA in presence of TTX (unpaired Student's *t*-test).

under basal conditions, the spontaneous activity of the portal vein does not appear to be under tonic control by NMDA receptors since (±)-AP-5 or CPP, when given alone, had little or no effect on vasomotor activity. NMDA at the concentration $10^{-3}$ M did not further increase the contraction frequency, but actually slightly reduced it as compared with the lower concentration of $10^{-4}$ M. Since washing returned the portal vein motility to control values, it is unlikely that excitotoxic effects play a major role in the observed response. Nonetheless, the contribution of non specific effects at this high concentration of NMDA cannot be ruled out.

After blockade of the intrinsic nerve activity of the portal vein by TTX, the excitatory effect of NMDA was reversed into a concentration-dependent inhibition of the contraction amplitude. The treatment with TTX alone did not significantly change the spontaneous phasic activity of the isolated rat portal vein, in agreement with previous studies in rats (Johansson & Ljung, 1967) and guinea-pigs (Ito & Kuriama, 1971). The inability of TTX to affect basal motility is also consistent with results showing that the local anaesthetic agent lidocaine, in concentrations which block impulse transmission in autonomic nerves, fails to interfere with conduction in the smooth muscle of the rat portal vein (Johansson & Ljung, 1967). Thus the NMDA-induced excitatory effect appears to be indirect and presumably mediated by excitatory neuronal afferents. However, the results with TTX are surprising since stimulation of pre-synaptic NMDA receptors would be expected to elicit neuronal activation and neurotransmitter release independent of sodium channel activity. Our results suggests, therefore, an axonal localization of NMDA receptors to afferent perivascular nerve fibres. In the central nervous system axonal localization of NMDA receptors has been already documented (Aoki *et al*., 1994; Gracy & Pickel, 1996). Electron microscopy studies have shown that NMDAR1-like immunoreactivity is localized to the extrasynaptic plasma membrane of neuronal processes, either dendrites or unmyelinated axons (Gracy & Pickel, 1996). Excitatory effects similar to those reported in our study were also observed in the longitudinal muscle myenteric plexus by Wiley *et al*. (1991), who showed that NMDA increases the contractile activity of the longitudinal smooth muscle in the ileum *via* the release of acetycholine. In the guinea-pig portal vein, Takata (1980) found that acetylcholine depolarized the membrane, increased the ionic conductance and the number and frequency of spike. Acetylcholine-induced contractions were also demonstrated in the guinea pig mesenteric vein (Takata, 1980). That a cholinergic mechanism might also account for the excitatory effects of NMDA observed in the present study is suggested by the ability of atropine to prevent the NMDA-induced excitation of contractile activity. Thus, the present results are consistent with the notion that the excitatory component of NMDA is indirectly mediated through a stimulation of cholinergic excitatory neuronal inputs.

On the other hand, the inhibitory effect of NMDA on the portal vein contraction activity, which is unmasked by the addition of TTX or atropine, is consistent with recent studies showing that NMDA receptors can modulate the contraction activity of cerebral microvessels and regulate cerebral blood flow (Fergus & Lee, 1997). In cerebral microvessels activation of NMDA receptors is accompanied by vasodilation, an effect analogous to that observed in the portal vein preparation of the present study. This vasodilatatory effect is apparently mediated by the formation of NO. NO has long been known to be a mediator of relaxation in smooth musculature (see Moncada *et al*., 1991 for review) and it is formed in the endothelium following activation of the constitutive form of the calcium-dependent and protein kinase-C regulated synthetising enzyme nitric oxide synthase (NOS) (Lamas *et al*., 1992). There is also evidence that increased calcium influx, secondary to activation of NMDA receptors, increases enzyme activity and NO formation in the CNS (see Schuman & Madison, 1994 for review). Our results show that the spontaneous contractile activity in the intact portal vein can be modulated by exogenous NO while removal of the endothelium abolishes the NMDA inhibitory response. Moreover, the ability of the NOS inhibitor L-NAME to abolishing the inhibitory component of NMDA observed in the presence of TTX further supports a role of NO in the NMDA effects. Thus, our results may indicate that endothelial NOS is involved in the NMDA-induced relaxation and suggest that the portal vein possesses NMDA receptors on the membrane of endothelial cells themselves. By activating these NMDA receptors, glutamate could trigger calcium influx, increase the formation of NO and elicit vasodilation.

On the other hand there is also considerable evidence for the presence of NO in inhibitory nerves innervating vascular tissues including penile, cerebral, mesenteric, coronary and pulmonary vessels (see Klimaschewski *et al*., 1992; Rand, 1992 for review) and neuronal as well as endothelial NO sources or other inhibitory transmitters (Brizzolara *et al*., 1993) can contribute to vasodilation. In the rat vasculature an equal distribution of NOS (neuronal – type I and endothelial isoforms) has been found on neuronal afferences and endothelial cells (Loesch *et al*., 1994; Loesch & Burnstock, 1994). Our results seem to indicate that in the rat portal vein it is the endothelial enzyme that is primarily coupled to NMDA receptor activation.

While further studies are required to confirm directly the presence of NMDA receptor subunits or their mRNAs in the portal vein, the present functional studies suggest that this vessel possess NMDA receptors that modulate its motor activity. Our results support the hypothesis of the existence of a peripheral excitatory glutamatergic system and suggest that disregulation of glutamatergic transmission may result in altered vasodilator responses.

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