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EFFECT OF NOR-TRIMEBUTINE ON NEURONAL ACTIVATION INDUCED BY A NOXIOUS STIMULUS OR AN ACUTE COLONIC INFLAMMATION IN THE RAT

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ABSTRACT

Nor-trimebutine is the main metabolite of trimebutine which is used in the treatment of patients with irritable bowel syndrome. Nor-trimebutine has a blocking activity on sodium channel and a potent local anaesthetic effect. These properties were used to investigate the effect of nor-trimebutine on spinal neuronal activation induced by models of noxious somato-visceral stimulus and acute colonic inflammation. Nor-trimebutine was administered subcutaneously in rats 30 min before intraperitoneal administration of acetic acid or intracolonic infusion of trinitrobenzenesulfonic acid. Abdominal contractions were counted for 1h as a marker of abdominal pain. *c-fos* expression was used as a marker of neuronal activation and performed by immunohistochemistry 1h after intraperitoneal acetic acid and 2h after colonic inflammation. Nor-trimebutine decreased Fos expression in the thoraco-lumbar (peritoneal irritation) and lumbo-sacral (colonic inflammation) spinal cord in laminae I, IIo V, VII and X. This effect was also observed in the sacral parasympathetic nucleus after colonic inflammation. Nor-trimebutine induced a significant decrease of abdominal contractions following intraperitoneal acetic acid. These data may explain the effectiveness of trimebutine in the therapy of abdominal pain in the irritable bowel syndrome.

Keywords

c-fos ; colitis ; somato-visceral pain ; trimebutine ; visceral afferents.

INTRODUCTION

Trimebutine (TMB) has been efficiently used in many countries since 1969 for the treatment of functional bowel disorders (Moshal et al., 1979; Lüttecke, 1980; Toussaint et al., 1981; Ghidini et al., 1986; Poynard et al., 2001), including irritable bowel syndrome (IBS), which is characterized by a visceral hypersensitivity (Ritchie, 1973). Besides its regulatory effects on colonic motility through its weak opioid properties, TMB is also able to influence the activity of visceral afferents (Julia et al., 1996; Roman et al., 1999) thus explaining its beneficial effects on abdominal pain in IBS patients. This effect is most likely due to its blocking activity on sodium channels (Roman et al., 1999). After oral administration in humans, TMB is metabolized in the liver to give nor-trimebutine (nor-TMB), the main metabolite of TMB, which reaches plasma levels higher than those of TMB itself (Roman et al., 1999). Nor-TMB has similar or more potent effects than TMB itself on sodium channel blockade and glutamate release inhibition (Lüttecke, 1980; Toussaint et al., 1981; Ghidini et al., 1986; Roman et al., 1999). This effect is of potential therapeutic interest not only in pain but also in inflammatory processing. Indeed, inflammation is well known to sensitise afferent fibers to pain (Bueno and Fioramonti, 1999) and IBS symptoms have been observed after infectious diarrhoea (Gwee et al., 1996) and in patients with inflammatory bowel diseases (IBD) in remission (Isgard et al., 1983). Intraperitoneal (ip) injection of acetic acid (AA) is a well known noxious chemical somato-visceral stimulus in rats (Koster et al., 1959; Menetrey et al., 1989; Hammond et al., 1992; Lanteri-Minet et al., 1993) as evidenced by the presence of abdominal contractions (Koster et al., 1959; Pairet et al., 1989; Rivière et al., 1993, 1994). Intracolonic (ic) instillation of trinitrobenzenesulfonic acid (TNBS) is a simple and reproducible model of colonic inflammation in rat (Morris et al., 1989). Fos, the protein product of the immediate early gene *c-fos* is a sensitive marker of neuronal activation classically used to localize

spinal cord neurons responsive to acute stimuli of somatic or visceral origin (Menetrey et al., 1989; Hunt et al., 1987; Herdegen et al., 1991; Traub et al., 1992, 1993). Using the model of somato-visceral pain, we have previously shown that, fedotozine, a kappa-opioid receptor agonist known to improve abdominal symptoms in IBS patients (Dapoigny et al., 1995; Delvaux et al., 1999), has an antinociceptive effect and prevents AA-induced Fos expression in the thoraco-lumbar spinal cord in rats through a peripheral action on visceral afferents (Bonaz et al., 2000).

In this study, we investigated in rats, the effects of nor-TMB on the induction of Fos expression in the spinal cord, i.e. the site of primary somato-visceral afferents, in the model of AA-induced somato-visceral pain and TNBS-induced acute colonic inflammation.

METHODS

Animals

Adult male Sprague-Dawley rats (250-300g; Charles River Laboratories, L'Abresle, France) were separately housed in a temperature-controlled environment (22±1°C) with food and water available ad libitum and a 12 hours on / 12 hours dark light cycle (lights at 8 a.m.). Animals were acclimatized to their environment for at least 7 days prior to the experiments. Because diurnal variations of Fos expression have been reported in some brain areas (Kononen et al., 1990), experiments were performed between 10:00 a.m. and 1:00 p.m. in animals deprived of food but not water for at least 18 hours. Protocols were conformed to the author's institution's animal care and use committee.

Noxious somato-visceral stimulus

Somato-visceral pain was induced in conscious rats (n=5) with ip injection of 0.6% AA (10 ml/kg) in the right lower quadrant of the abdomen as previously described (Bonaz et al., 2000). Control animals (n=5) received the vehicle alone (saline ip: 10 ml/kg). Somato-visceral pain was assessed by counting abdominal contractions (Koster et al., 1959; Giamberardino et al., 1995; Bonaz et al., 2000) for 60 minutes, by periods of 10 min, following the ip injection of AA, classically described as the writhing test (Koster et al., 1959). Abdominal contractions consisted of the contraction of the flank muscles associated with inward movements of the hindlimb or with whole body stretching (Giamberardino et al., 1995).

Acute colonic inflammation

Rats were anesthetised with a mixture of sodium pentobarbital and chloralhydrate (4 ml/kg ip) (Bonaz et al., 2000). A silicone catheter (ID, 1.2 mm; OD, 1.7 mm) was chronically implanted into

the distal colon 7-8 cm from the anus. The catheter was fixed at the colonic wall by a purse-string suture, tunnelled subcutaneously and externalised at the back of the neck where it was secured at the animal's skin. After surgery, animals were housed separately for at least 7 days prior to testing. Experiments were performed in conscious freely moving rats equipped with this chronic catheter to avoid the possible confounding effects of stress-induced manipulation of the animals on c-fos expression (Kovacs, 1998). Inflammation of the colon was induced through the catheter with a single ic administration of 0.25 ml of 50% ethanol containing 30 mg of TNBS (Fluka, St Quentin Fallavier, France) as previously described (Morris et al., 1989). The instillation procedure required ~ 5s to complete. Catheter was then slowly flushed with 0.2 ml air to expel any inducing agent remaining in the catheter. Control animals received 0.25 ml of 0.9% saline according to the same procedure. Assessment of colonic damage was performed after sacrifice of the animals for Fos-immunohistochemistry processing. After completion of the perfusion, the distal colon was isolated, opened by a longitudinal incision, rinsed with saline and then pinned out on a wax block and examined macroscopically.

Drugs

In the model of somato-visceral pain, nor-TMB (10 or 30 mg/kg subcutaneously (sc) dissolved in 250 µl saline; n=5 in each group) or the vehicle (250 µl saline subcutaneously; n=5) was administered 30 min before ip injection of AA. The effect of nor-TMB (10 or 30 mg/kg in 250 µl saline subcutaneously; n=5) alone on Fos expression was also investigated 90 min after injection.

In the model of acute colonic inflammation, nor-TMB (30 mg/kg dissolved in 250 µl saline; n=5) or the vehicle (250 µl saline; n=5) was administered ic, 30 minutes before induction of colitis. The dose of 30 mg/kg was chosen based on the somato-visceral pain procedure showing that this dose

decreased abdominal contractions counted during 60 min. The effect of ic injection of nor-TMB alone (n=3) on Fos expression was also investigated 2h30 after administration of the compound.

Fos immunohistochemistry

Fos immunohistochemistry was performed as previously described (Bonaz et al., 1994, 2000). Sixty min after ip AA or 2 hours after intracolonic TNBS, rats were sacrificed. The 1h-interval after ip AA was selected based on previous studies (Bonaz et al., 1994; 2000). The 2h-interval after ic instillation of TNBS was selected based on a time course effect on Fos expression in the spinal cord in preliminary studies (personnal unpublished data). Animals were deeply anesthetised with an ip injection of a mixture of sodium pentobarbital and chloralhydrate (4 ml/kg ip) and then transcardially perfused with 100 ml of isotonic saline followed by 600 ml of 4% paraformaldehyde in 0.1M phosphate buffer (PB; pH 7.4). Spinal cords were rapidly removed, postfixed for 3 hours at +4°C in the same fixative and subsequently cryoprotected overnight in 20% sucrose in 0.1M PB. Coronal frozen sections (30 μm) of the spinal cord (thoracic, lumbar and sacral levels) were cut on a cryostat (Microm, Lyon, France) and processed for Fos-IR. Free-floating sections were incubated for 16-18 hours at +4°C with the primary antibody (Fos AB-5 rabbit polyclonal antibody, Oncogen Science; dilution 1:1000 in PB saline, 0.02M, containing 0.5% Triton X-100 and 10% normal goat serum) and then with a biotinylated secondary antibody (goat anti-rabbit, Sigma, St Louis, MO; dilution 1:100) for 2 hours at room temperature. Sections were finally processed for avidin-biotin-peroxidase using diaminobenzidine as the chromogen (Sigma), then mounted on gelatin-coated slides, dehydrated, cleared in xylene, and coverslipped.

Fos quantification. The presence of Fos immunoreactivity (Fos-IR) was detected by brightfield microscopy as a brown-black reaction product in cell nuclei. No Fos-IR was observed in sections when the primary antibody was omitted. Fos count was performed as previously described (Bonaz

et al., 1994, 2000). In the model of somato-visceral pain, Fos positive cells were counted at the thoraco-lumbar level (T10-L2) of the spinal cord as previously described (Bonaz et al., 2000) on 20 consecutive sections, in specific laminar regions of the spinal gray matter i.e. laminae I-outer II (IIo), V, VII and X, using the cytoarchitectonic organization of the spinal cord described by Molander (Molander et al., 1984, 1989). In the model of acute colonic inflammation, Fos-IR was counted on 20 consecutive sections at the lumbo-sacral level (L6-S2) in the same laminae as well as in the sacral parasympathetic nucleus (SPN) at S1 level. To estimate the total number of neurons, counts were corrected for double-counting errors by the formula of Abercrombie (1946): corrected count = total count x (section thickness/average diameter of neurons + section thickness). In both models, Fos count was expressed as either total (laminae I-IIo+V+VII+X) or individual (lamina I-IIo, V, VII, X). Cell counting was performed in blind conditions to avoid bias.

Statistical analysis

Data are expressed as means ± standard error of the mean (S.E.M). Difference between means of Fos-positive cells within different spinal segments were determined by a variance analysis (ANOVA). Post hoc comparison between groups was evaluated by the Tukey test. Data were considered statistically significantly when $P<0.05$.

RESULTS

Effect of nor-TMB on acetic acid-induced abdominal contractions

In control animals, almost no abdominal contraction was observed while a dramatic increase of abdominal contractions was counted during visual observation of the animals for 60 minutes following AA ip (Fig 1A). *Nor-TMB*, administered at a dose of 10 or 30 mg/kg sc, did not significantly modify the number of AA-induced abdominal contractions when considering the overall period of 60 min while a non significant decrease of abdominal contractions was observed with the dose of 30 mg/kg (Fig 1A). In contrast, a significant decrease of abdominal contractions was observed for both doses when considering the last 2 periods of 10 minutes (from 40 to 60 min) following AA ip (Fig 1B).

Effect of nor-TMB pretreatment on acetic acid-induced Fos-IR

In control animals receiving 250µl saline sc 30 min before 10 ml/kg saline ip, almost no Fos-IR was observed in the spinal cord, as previously described (Bonaz et al., 2000) (data not shown). *Animals pre-treated sc with saline and then with ip AA* presented the same pattern of Fos-IR than previously described (Bonaz et al., 2000). Fos labeling was essentially distributed at the thoraco-lumbar level (T10-L2) mainly in the superficial dorsal horn (lamina I-IIo) but also in laminae V, VII and in the region surrounding the central canal i.e. lamina X (Fig2A, Figs 3A,B). These neurons were predominantly concentrated on the side ipsilateral to the injection site although they were also present in reduced numbers on the controlateral side. Few Fos-IR neurons were observed in laminae III and IV. A few labelled cells were observed in the region of the intermediolateral cell column. The number of Fos positive neurons in the different laminae is detailed in Figs 3 A,B. Almost no Fos-IR was observed at the cervical, upper thoracic and sacral levels (data not shown). *Nor-TMB* (10 or 30mg/kg sc) *administered alone* did not induced any significant Fos-IR in the

spinal cord (data not shown). *Nor-TMB at a dose of 10 or 30 mg/kg sc* significantly decreased total Fos count in AA-treated rats (Fig2B, Figs 3A,B). The significant decrease of Fos count for the dose of 10 mg/kg sc was 52%, 74.5%, 79%, 78% in laminae I-IIo, V, VII, and X respectively (Fig 3B). The higher dose (30 mg/kg sc) of nor-TMB produced a similar effect: 59%, 79%, 79% and 83% in laminae I-IIo, V, VII, and X respectively (Fig 3B).

Effect of nor-TMB pre-treatment on acute colonic inflammation-induced Fos-IR

Ic administration of 0.9% saline alone did not produce any behavioural modification of the animals as well as any macroscopically detectable damage of the recto-colon. *All animals receiving TNBS/ethanol* showed reduced fluid and food intake, diarrhoea, piloerection, lack of preening, and a reduced level of activity during the 2h following induction of colitis. Ic administration of TNBS did not induced any abdominal contraction. Extensive macroscopic damage of the colon was observed in all animals at the site of TNBS instillation. The mucosa appeared ulcerated and haemorrhagic. The site of inflammation generally involved the rectum until the splenic flexure. Damage was rarely observed before the splenic flexure. No macroscopic abnormality of the liver, spleen, kidney, ileum, jejunum or duodenum was observed.

In control animals receiving 250µl saline ic 30 min before 250µl saline ic, few Fos-IR was observed in the spinal cord (Fig 4A). *In animals receiving ic injection of saline and TNBS 30 min later,* a strong Fos-IR was observed in the spinal cord, predominantly in L6-S2 segments. Fos-IR was distributed in laminae I-IIo, V, VII and X and in the SPN (Fig2C, Figs 4 A,B). A very few Fos IR was observed at the thoracolumbar level (T10-L2) (data not shown). *Ic injection of nor-TMB alone* did not induced any Fos-IR in the spinal cord (data not shown). *In animals pretreated with nor-TMB (30 mg/kg ic)* 30 min before TNBS injection, a significant decrease of total Fos count was observed in the spinal cord at L6-S2 segments and in the SPN (Fig2D, Fig 4A). This decrease was

62% ($P<0.05$), 48% (NS), 66% (NS), 78% ($P<0.05$) and 75% ($P<0.05$) in laminae I-IIo, V, VII, X and in the SPN respectively (Fig 4B).

DISCUSSION

The present study shows that nor-TMB, the main metabolite of TMB, used in the treatment of IBS, is able to decrease neuronal activation induced in the spinal cord by a noxious somato-visceral stimulus or an acute colonic inflammation.

Nor-TMB is the major circulating compound after oral administration of TMB in humans and has similar or more potent effects than TMB itself (Roman et al., 1999). TMB, nor-TMB and their corresponding stereoisomers inhibit veratridine-induced glutamate release in vitro (Roman et al., 1999). Glutamate is an excitatory amino acid known to mediate spinal transmission of peripheral nociceptive input (Scatton, 1993; Urban et al., 1994), by activating voltage-dependent sodium channels (Wermelskirchen et al., 1992). The effect of TMB and nor-TMB is not due to their opioid properties but to their blocking activities on voltage-dependant sodium channels (Roman et al., 1999) which are expressed in primary sensory neurons and are important targets in the study of the molecular pathophysiology of pain (Waxman et al., 1999). The sodium channel blocking activity is confirmed by the potent local anaesthetic effect of TMB which is 17-fold more active than lidocaine (Roman et al., 1999), an effective drug in the treatment of human and experimental colitis (Bjorck et al., 1989, 1992). TMB and/or nor-TMB may influence activity of visceral afferents in rats as represented by an inhibition of the rectocolonic reflex (Julia et al., 1996) and rectal hyperalgesia evoked by TNBS-induced colitis and stress in rats (Lacheze et al., 1998), as well as by a blockade of sodium currents in sensory neurons from rat dorsal root (Roman et al., 1999). Nor-TMB has also an antinociceptive effect in a model of neuropathic pain in rats (Kayser et al., 2000). The properties of TMB and nor-TMB may explain the effectiveness of TMB in the therapy of abdominal pain in IBS patients (Lüttecke, 1980; Toussaint et al., 1981; Ghidini et al., 1986; Poynard et al., 2001).

In the model of somato-visceral stimulus, neuronal activation induced by AA ip was observed in the same locations and laminae of the spinal cord as we (Bonaz et al., 2000) and others (Menetrey et al., 1989; Hammond et al., 1992; Lanteri-Minet et al., 1993) have previously described. Indeed, Fos-IR was observed in laminae I, IIo, V, and X, reported to receive unmyelinated (C) as well as fine myelinated (aδ) somato-visceral afferents (Neuhuber, 1982, 1986; Sugiura et al., 1989) while Fos-IR cells observed in lamina VII most likely corresponds to polysynaptically activated neurons (Hammond et al., 1992; Bonaz et al., 2000). Lamina VII has been shown to receive projections from muscle nerves in the rat (Molander and Grant, 1987). Moreover, some cells of lamina VII are part of the spinoreticular tract, one of the ascending nociceptive pathways. Laminae I, IIo, V, VII and X are important regions for rostral transmission of nociceptive information to a number of sites which are involved in homeostasis and visceral integration (Menetrey and Basbaum, 1987; Menetrey and Pommery, 1991; Giesler et al., 1994; Al-Chaer et al., 1996, 1997; Bester et al., 1997; Bourgeais et al., 2001) and underly the production or modification of neuroendocrine, autonomic, affective and emotional responses to painful stimuli (Berthier et al., 1988; Yasui et al., 1991; Katter et al., 1996; Zhang et al., 1999). The few Fos-IR observed in laminae III-IV is most likely induced by the motor activity of abdominal contractions due to peritoneal irritation. Lamina III contains numerous fine myelinated fibers, but many cells respond only to weak mechanical stimuli (Cervero, 1988). Lamina IV cells respond to proprioceptive and cutaneous stimuli (Matsushita et al., 1979) and also only to light mechanical stimuli (Cervero, 1988). The effect of nor-TMB in decreasing neuronal activation was not dose-dependent since the decrease of Fos-IR in the spinal cord was in the same order of magnitude either with 10 or with 30 mg/kg sc. We did not test a smaller dose than 10 mg/kg since experimental data have shown that TMB is active on abdominal pain from 10 mg/kg (Julia et al., 1996). The antinociceptive effect of nor-TMB was confirmed by a significant decrease of abdominal contractions as observed from 40 to 60 min following AA ip. These data are

close to the ones we have observed with fedotozine (Bonaz et al., 2000), a kappa opioid agonist able to improve abdominal symptoms in IBS patients (Dapoigny et al., 1995; Delvaux et al., 1999). In the present study, the selection of a 1h-interval after AA ip was based on previous studies (Bonaz et al., 1994, 2000); this is also the optimal time interval to reveal Fos expression after exposure to various types of stimuli (Morgan and Curran, 1990).

In the model of acute colonic inflammation, the localization of Fos-IR in the lumbosacral (L6-S2) and at a lesser extent in the thoraco-lumbar segments of the spinal cord is in agreement with the topographical distribution of afferent projections from the colon and rectum (Ness and Gebhart, 1990). At L6-S2 segment, Fos was located primarily in the superficial layers of the dorsal horn (i.e. laminae I-IIo), in lamina VII, in area X surrounding the central canal, and in the SPN. This distribution matches the rostro-caudal and topographical central projections of pelvic afferents established to terminate in laminae I, IIo, V, VI and VII, and the dorsal part of area X (Morgan et al., 1981; Nadelhaft and Booth, 1984; De Groat, 1986; Pascual et al., 1989). This distribution is also in agreement with the one observed after colorectal (Traub et al., 1992, 1993) or proximal colon distension (Martinez et al., 1998), as well as after electrical stimulation of the pelvic nerve (Birder et al., 1991) or after chemical irritation of the lower urinary tract (Birder et al., 1999). Few Fos-IR was also observed in the thoraco-lumbar spinal cord as observed by Traub et al. (1992, 1993). Nor-TMB decreased Fos-IR in laminae I-IIo, V, VII, and X, as well as in the SPN, with a significant effect in lamina I-IIo, X and the SPN while the decrease was nearly significant in laminae V and VII. The parasympathetic preganglionar pathway to the pelvic organ arises in the L6-S1 spinal segments in the rat (Nadelhaft and Booth, 1984). The SPN has an important role in the regulation of various pelvic organ functions including micturition, defecation and penile erection (De Groat et al., 1996). Neurons in the SPN have extensive axon collaterals with abundant synaptic connections with neurons in area X surrounding the central canal, but also

with neurons in laminae I, V, VII and IX (Morgan et al., 1991). The activation of neurons within the SPN could thus participate, at least in part, to the Fos-IR observed around the central canal (laminae X). Mawe et al (1986) have also shown connections between primary sacral afferent fibers and dendrites of labelled neurons in the SPN. These connections thus provide evidence for sensory and autonomic reflex integration and could explain the induction of Fos expression in the SPN and the preventive effect of nor-TMB on Fos-IR in the SPN. In this model, only one dose of nor-TMB (30 mg/kg) was used, based on the somato-visceral pain procedure showing that this dose decreased abdominal contractions counted during 60 min.

In the present study, nor-TMB has been administered by two different routes (sc and ic). Consequently, the results may reflect different sites and modes of action of nor-TMB. Indeed, we may evoke: 1) A possible blocking activity effect on somato-visceral afferents through the properties of the drug (Roman et al., 1999). An influence of the compound on visceral afferents and dorsal root ganglia culture neurons has been observed in rats (Julia et al., 1996; Roman et al., 1999; Lacheze et al., 1998), 2) An anti-inflammatory action in the model of acute colonic inflammation, through the potent local anaesthetic properties of the compound on visceral afferents (Roman et al., 1999). One might argue that second or third order neurons are activated in the spinal cord, all of which receive modulatory descending influences suggesting a role in the descending control of pain. We did not perform *c-fos* expression in spinal transected animals to be sure that the distribution of Fos positive neurons was similar in spinal intact and spinal transected animals thus indicating that *c-fos* expression was mediated by monosynaptic afferent input or input from segmental interneurons and was not due to activation of supraspinal pathways. However, it has been shown that in spinally transected rats the induction of Fos-IR was significantly increased 3 days, but not 2 h, following inflammation (Ren and Ruda, 1996). In the present study, *c-fos* expression was performed 1h or 2h after peritoneal or colonic inflammation

respectively. Although nor-TMB administered alone sc or ic did not induce any significant Fos-IR in the spinal cord, one can wonder that this does not rule out a central effect of the drug. However, it has been shown that the stimulating effect of TMB on intestinal motility (phase 3) is not reproduced after intracerebroventricular administration and is abolished by previous intravenous, but not intracerebroventricular, administration of naloxone (Honde et al., 1989). One can wonder that nor-TMB was given systemically (sc) and locally (ic) at times before AA or TNBS and sacrifice that do not prevent nor-TMB from accessing all of the neuraxis, including afferent and efferent fibers, spinal cord neurons and supraspinal sites (not explored in the present study). However, this time course is the one generally used in experimental studies on visceral pain with TMB and nor-TMB (Julia et al., 1996; Lacheze et al., 1998). Despite the two different routes of administration of the compound, the same laminae of the spinal cord, mostly involved in somato-visceral processing, were activated.

In the present study, we were only looking at the acute effects of a noxious or inflammatory insult. However, experimental data have also shown an effect of TMB and nor-TMB on rectal hyperalgesia induced by local inflammation and stress (Lacheze et al., 1998); these effects might explain the efficacy of TMB in the treatment of IBS. Inflammation is well known to sensitize afferent fibers to pain (Bueno and Fioramonti, 1999) and symptoms of IBS have been observed in patients with IBD in remission (Gwee et al., 1996). Consequently, TMB could also be of potential interest in treating abdominal pain either in IBD or in IBD patients with IBS symptoms.

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FIGURE LEGENDS

Figure 1

A: Abdominal contractions counted during visual observation of the animals for 60 min following 250µl saline subcutaneously (sc) 30 min before 10 ml/kg saline intraperitonealy (ip; controls) or 10 ml/kg 0.6% acetic acid (AA) ip (vehicle+AA), and in animals pre-treated sc 30 min before ip AA with 10 or 30 mg/kg nor-TMB dissolved in 250µl saline (nor-TMB+AA). NS: non significant. **B** : Abdominal contractions counted by periods of 10 min during visual observation of the animals for 60 min following 10 ml/kg 0.6% AA ip in animals pre-treated sc 30 min before with 250µl saline (vehicle+AA), and in animals pre-treated sc 30 min before ip AA with 10 or 30 mg/kg nor-TMB dissolved in 250µl saline (nor-TMB+AA). *$P<0.05$ vs. vehicle+AA-treated animals**.**

Figure 2

Distribution of Fos-immunoreactive cells in 1) the *lower thoracic level (T12)* of the spinal cord following 0.6% ip acetic acid (AA) in animals pre-treated subcutaneously (sc) 30 min before with sc 250µl saline (vehicle+AA) (**A**), or sc 30 mg/kg nor-TMB dissolved in 250µl saline (nor-TMB+AA) (**B**); 2) the *lower lumbar level (L6)* of the spinal cord following 250 µl of 50% ethanol containing 30 mg of trinitrobenzenesulfonic acid intracolonicaly (ic) in animals pre-treated ic 30 min before with 250µl saline (vehicle + colitis) (**C**), or 30 mg/kg nor-TMB dissolved in 250µl saline (nor-TMB+colitis) (**D**). I-IIo: lamina I-IIo; V: lamina V; VII: lamina VII; X: lamina X; SPN: sacral parasympathetic nucleus. cc: central canal. Scale bar: 50µm for Fig 2 A,B,C ; 80µm for Fig 2 D.

Figure 3

A: Fos positive cells (mean nb/hemisection) in total laminae (I-IIo, V, VII and X) of the thoraco-lumbar (T10-L2) spinal cord segment in animals receiving 250µl saline subcutaneously (sc) 30 min before 10 ml/kg saline intraperitonealy (ip; controls) or 10 ml/kg 0.6% acetic acid (AA) (vehicle+AA), and in animals pre-treated sc 30 min before ip AA with 10 or 30 mg/kg nor-TMB dissolved in 250µl saline (nor-TMB+AA). *P<0.05 vs. vehicle+AA-treated animals. **B**: Laminar distribution (mean nb/hemisection) of Fos positive cells in the thoraco-lumbar (T10 -L2) spinal cord in animals receiving 250µl saline sc 30 min before 10 ml/kg saline ip (controls) or 10 ml/kg 0.6% AA (vehicle+AA), and in animals pre-treated sc 30 min before ip AA with 10 or 30 mg/kg nor-TMB dissolved in 250µl saline (nor-TMB+AA). *P<0.05 vs. vehicle+AA-treated animals.

Figure 4

A: Fos-positive cells (mean nb/section) in total laminae (I-IIo+V+VII+X) and in the sacral parasympathetic nucleus (SPN) of the lumbosacral (L6-S2) spinal cord in animals receiving 250µl saline subcutaneously (sc) 30 min before 250µl saline intracolonicaly (ic; controls) or 250µl ic of 50% ethanol containing 30 mg of trinitrobenzenesulfonic acid (TNBS) (vehicle+colitis), and in animals pre-treated ic 30 min before colitis with 30 mg/kg nor-TMB dissolved in 250µl saline (nor-TMB+colitis). *P<0.05 vs. vehicle+colitis-treated animals. **B**: Laminar distribution (mean nb/section) of Fos positive cells in laminae I-IIo, V, VII, X and in the SPN of the lumbosacral (L6-S2) spinal cord in animals receiving 250µl saline sc 30 min before 250µl saline ic (controls) or 250µl ic of 50% ethanol containing 30 mg of TNBS (vehicle+colitis), and in animals pre-treated ic 30 min before colitis with 30 mg/kg nor-TMB dissolved in 250µl saline (nor-TMB+colitis). *P<0.05 vs. vehicle+colitis-treated animals.