Effects of L-Arginine, L-NAME, Methylene Blue, and Their Combinations on Corchorus olitorius Aqueous Extract Antinociception in Mice

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Abstract

The current study was carried out to investigate the possible involvement of the L-arginine/nitric oxide/cyclic guanosine monophosphate (L-arginine/NO/cGMP) pathway in the aqueous extract of Corchorus olitorius L. (Tiliaceae) (AECO) antinociception in mice, assessed by the abdominal constriction test. The AECO, obtained by soaking the dried leaves in distilled water (dH2O) (1:2; w/v) for 24 h, was prepared at concentrations of 10%, 50%, and 100% and administered subcutaneously (s.c.) 5 min after pretreatment (s.c.) of mice with dH2O, L-arginine (20 mg/kg), L-NAME (20 mg/kg), and methylene blue (MB) (20 mg/kg), respectively. AECO was found to exhibit concentration-independent antinociception with 100% AECO showing total loss of activity. Pretreatment with 20 mg/kg L-arginine, 20 mg/kg Nω-nitro-L-arginine methyl esters (L-NAME), or 20 mg/kg methylene blue (MB) was found to enhance the 50% AECO antinociception while significantly improving the ineffective 10% and 100% AECO antinociception. Meanwhile, L-arginine reversed, MB enhanced, and L-NAME produced insignificant changes in 10 mg/kg acetylsalicylic acid (ASA) antinociception. Cotreatment with L-NAME, significantly (p < 0.05) reversed the L-arginine effect only on 100% AECO antinociception. However, the antinociceptive activity was still maintained. Cotreatment with MB, on the other hand, suppressed the L-arginine effect on ASA and 10% AECO while increasing its effect on the 50% and 100% AECO. In conclusion, this study has demonstrated the involvement of the L-arginine/NO/cGMP pathway in AECO antinociception.

Keywords: Abdominal constriction test, antinociception, aqueous extract, Corchorus olitorius, L-arginine, L-NAME, methylene blue.

Introduction

Corchorus olitorius L. (Tiliaceae) is used as an herbal medicine and is eaten as a vegetable by local people in various parts of the world, including Egypt, India, the Phillipines, and Malaysia (Zeghichi et al., 2003). Known as “senaung betina” to the Malaysian’s Sabahan people, the leaves and roots of C. olitorius are used traditionally as a demulcent, diuretic, febrifuge, and tonic, and also in the treatment of pain and fever (Abu-Hadid et al., 1994; Zeghichi et al., 2003). Previous studies have demonstrated estrogenic activity with the seed oil of C. olitorius (Sharaf et al., 1979). On the other hand, the seeds of C. olitorius were also reported to contain high content of hydrogen cyanide and several cardiac glycosides (Negm et al., 1980). In addition, C. olitorius extracts were also found to exhibit weak and moderate inhibitory effects toward the mutagenicity of benz(a)pyrene or 2-amino-3-methyl-imidazo[4,5-f]quinoline in Salmonella typhimurium TA98 and TA100, respectively (Yen et al., 2001).

Gupta et al. (2003) have recently reported on the anticonvulsive properties of a methanol extract of C. olitorius seed in mice, and have suggested that the observed effect might be due to its ability to alter...
brain amino acids and catecholamine levels in mice. In addition, there is also a report on the ability of *Corchorus olitorius* leaf extract to reduce elevation of postprandial blood glucose levels in rats as well as humans (Innami et al., 2005). It was also suggested that this observation is attributed to the viscous soluble dietary fiber ability of the leaves to delay glucose absorption from the intestinal membrane in the upper digestive tract. To support the above-mentioned finding, Ohtani et al. (1995) successfully isolated an acidic polysaccharide from the water-soluble mucilage extract of dried leaves of *Corchorus olitorius*. The authors have also reported that the isolated polysaccharide exhibited proliferative activity toward the murine splenocyte (Ohtani et al., 1995).

Recently, we have reported the peripherally and centrally mediated antinociceptive activities of aqueous extract of *Corchorus olitorius* (AECO) leaves (Zakaria et al., 2005), which is mediated, at least in part, via the opioid receptor. Furthermore, these activities were also found to resist the effect of extreme temperature (Zakaria et al., 2005). Apart from that, we have also demonstrated the involvement of a number of receptors, like *μ*-opioid, GABA, *α* - and *β*-adrenergic, and nicotinic receptors, at the peripheral level in the AECO antinociceptive activity, as assessed using the abdominal constriction test. This peripheral activity, which decreased significantly under alkaline conditions or when pretreated with *α*-amylase, was found to be maintained under acidic conditions or when pretreated with protease or lipase (data not published), respectively. The ability of *α*-amylase to reverse *Corchorus olitorius* antinociceptive activity suggested the presence of a polysaccharide-based bioactive compound. Whether the bioactive compound is purely a polysaccharide or contains polysaccharide as part of its active compound needs further study. These findings were in line with a report by Ohtani et al. (1995) as mentioned earlier. However, investigation on the above-mentioned parameters on centrally mediated antinociceptive activity of AECO is still being carried out in our laboratory.

Based on a literature search, we were unable to find any scientific reports on *Corchorus olitorius* antinociceptive and other pharmacological activities, other than those mentioned above, which seems to indicate lack of exploration on the pharmacological activities of this plant despite the traditional claims described earlier. Based on that fact and on our recent findings, we decided to take this opportunity to study the involvement of the *L*-arginine/nitric oxide/cyclic guanosine monophosphate (*L*-arginine/NO/cGMP) pathway in *Corchorus olitorius* antinociception by focusing on its peripherally mediated mechanism.

### Materials and Methods

#### Plant material

*C. olitorius* leaves were collected by Mrs. Fatimah Corazon Abdullah from the district of Shah Alam, Selangor, Malaysia, between January and February 2005, and a voucher specimen (SK 963/04) was deposited at the herbarium of the Institute of Bioscience, Universiti Putra Malaysia (UPM), Malaysia.

#### Preparation of *Corchorus olitorius* leaf aqueous extract (AECO)

Preparation of the *Corchorus olitorius* aqueous extract of leaves was carried out according to the method described by Zakaria et al. (2005). The leaves were rinsed with water and then oven-dried for 72 h at the temperature of 50°C. The dried leaves were then ground into small particles, weighed distilled water (dH₂O) added in the ratio of 1:25 (v/w). This mixture was then left for 24 h, and the supernatant was collected and filtered using Whatman no. 1 filter paper while the remaining plant residue was kept in an oven for future use. The supernatant obtained, labeled as AECO and considered as stock solution with 100% concentration/strength, was diluted with dH₂O to the concentration of 10% and 50%, and used together in the antinociceptive study.

#### Preparation of drugs

Acetylsalicylic acid (ASA) (Bayer, Singapore), used as positive control group, was prepared in the dose of 100 mg/kg, while *L*-arginine (Sigma, St. Louis, MO, USA), *N*-nitro-*L*-arginine methyl esters (*L*-NAME; Sigma), and methylene blue (MB; Sigma) were prepared in the dose of 20 mg/kg (Jain & Kulkarni, 1999; Abacioglu et al., 2000) by dissolving them in distilled water (dH₂O).

#### Experimental animals

Male Balb-C mice (25–30 g; 5–7 weeks old) were used in this study and kept under room temperature (27 ± 2°C; 70–80% humidity; 12-h light/dark cycle) in the Animal Holding Unit (UPM) for at least 48 h before used. Food and water were supplied *ad libitum* up to the beginning of the experiments. At all times, the experimental procedures were carried out in strict compliance with the animal ethics committee rules and regulation followed by the university and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). All experiments were conducted between 0930 and 1230 h to minimize the effects of environmental changes (Mat Jais et al., 1997).
All mice were divided into 25 groups of 10 mice each (n = 10). Five groups were pretreated with dH₂O followed 5 min later by treatment with dH₂O, ASA (10 mg/kg), or AECO (10%, 50%, and 100% concentration), respectively. The remaining 20 groups were pretreated with 20 mg/kg L-arginine, D-arginine, L-NAME, or MB followed 5 min later by treatment with dH₂O, ASA, or AECO (10%, 50%, and 100% concentration), respectively. Thirty minutes after administration of the respective DH₂O, ASA, or AECO, the mice were injected with 0.6% acetic acid intraperitoneally (i.p.) (Mat Jais et al., 1997; Dambisya et al., 1999). All drugs and solutions were administered subcutaneously (s.c.) in the volume of 10 ml/kg.

Antinociceptive assay

The abdominal constriction test described by Dambisya and Lee (1995) was used to investigate the involvement of the L-arginine/NO/cGMP pathway in AECO-induced antinociception. Briefly, acetic acid (J.T. Baker, Phillipsburg, NJ, USA), prepared as 0.6% (v:v) solution in dH₂O and used to induce pain in the mouse peritoneal cavity, was administered i.p. (10 ml/kg of mice) 30 min after the s.c. administration of respective dH₂O, ASA, or AECO. The abdominal constriction or writhing response resulting from the injection of acetic acid consisted of a contraction of the abdominal muscle together with a stretching of the hind limbs (Duarte et al., 1990). The number of abdominal constrictions was counted cumulatively over the period of 25 min, 5 min after the acetic acid administration. Antinociception was calculated using a formula described by Dambisya and Lee (1995) as the percentage inhibition of abdominal constrictions (percentage of inhibitory level) using the formula

\[
\left(\frac{\text{saline control group mean} - \text{test group mean}}{\text{saline control group mean}}\right) \times 100\%
\]

and presented in the form of histogram.

Statistical analysis

The results obtained were analyzed using one-way analysis of variance (ANOVA) test followed by Dunnett post test, with \( p < 0.05 \) as the limit of significance.

Results

The antinociceptive profile of ASA and AECO pretreated with dH₂O was shown in all histograms described later for the purpose of comparison with those pretreated with the respective drugs. The AECO was found to show concentration-independent antinociception with only the 50% concentration extract producing an activity that is significant (\( p < 0.05 \)) when compared with the control group.

Figure 1 shows a comparison between the antinociception of AECO pretreated with dH₂O or L-arginine (NO donor). L-Arginine (20 mg/kg) was found to induce significant (\( p < 0.05 \)) nociception when pretreated with dH₂O, reversing the ASA antinociception. Interestingly,
L-arginine was found to significantly \((p < 0.05)\) improve the AECO activity, at all concentrations used. Pretreatment with L-NAME (NO inhibitor), with either dH\(_2\)O or with ASA, caused insignificant change in the number of abdominal constrictions (Fig. 2). On the other hand, L-NAME was also found to significantly \((p < 0.05)\) enhance the antinociceptive activity of all concentrations of AECO.

\[\text{Combination treatments and Corchorus} \]

As can be seen in Figure 3, pretreatment with MB (cGMP inhibitor) followed by dH\(_2\)O was found to produce significant \((p < 0.05)\) antinociception, while pretreatment with ASA or AECO, at all concentrations, was found to significantly enhance \((p < 0.05)\) antinociceptive effects.

Figure 4 shows a comparison between the effects of pretreatment with L-arginine, L-NAME, or their
combination (L-arginine + L-NAME) on ASA and AECO antinociception. There is an insignificant increase in the number of abdominal constrictions in the group pretreated with (L-arginine + L-NAME) when compared with the control group (dH2O + dH2O). Furthermore, ASA activity, which was reversed by L-arginine, increased insignificantly when pretreated with (L-arginine + L-NAME). Except for the 100% concentration AECO, pretreatment with (L-arginine + L-NAME) was found to produce an antinociceptive activity in 10% and 50% concentrations AECO that is insignificant when compared with the same group pretreated only with

\[ \text{Figure 4. Effects of L-arginine, L-NAME, or its combination (L-arginine + L-NAME) on ASA and AECO antinociception assessed by abdominal constriction test.} \]

\[ \text{a, differ significantly (p < 0.05) when compared against the control [(dH}_2\text{O + dH}_2\text{O)-treated] group; b, c, d, differ significantly (p < 0.05) when compared against the respective [(dH}_2\text{O)-pretreated] group; e, differ significantly (p < 0.05) when compared against the respective [(l-arginine)-pretreated] group.} \]

\[ \text{Figure 5. Effects of L-arginine, MB, or its combination (L-arginine + MB) on ASA and AECO antinociception assessed by abdominal constriction test.} \]

\[ \text{a, differ significantly (p < 0.05) when compared against the control [(dH}_2\text{O + dH}_2\text{O)-treated] group; b, c, d, differ significantly (p < 0.05) when compared against the respective [(dH}_2\text{O)-pretreated] group; e, differ significantly (p < 0.05) when compared against the respective [(l-arginine)-pretreated] group.} \]
L-arginine. For the 100% concentration AECO, the activity after treatment with (L-arginine + L-NAME) was significantly reversed when compared with the same group pretreated only with L-arginine.

Figure 5 illustrates the effects of pretreatment with (L-arginine + MB) on antinociceptive profiles of dH2O, ASA, and AECO compared with the respective group pretreated with only dH2O, L-arginine, or MB. Interestingly, MB was found to significantly (p < 0.05) reverse the L-arginine-induced nociceptive effect while improving the antinociceptive effect of AECO given alone. Although (L-arginine + MB) produced antinociception on its own, maintaining the ASA effect while improving the 50% concentration AECO antinociception, and reversing the 10% and 100% AECO lack of effect when compared with the respective group pretreated with only L-arginine, there is a significant (p < 0.05) reduction in activity if compared with the group treated only with MB.

Discussion

NO has been reported to be involved in the mechanism of nociception (Duarte et al., 1990) as either a pronociceptive (at low concentration) or an antinociceptive agent (at higher concentration) (Ferreira et al., 1991; Kawabata et al., 1994). These activities are peripheral, which involves a more complex mechanism of nociceptive transmission (Duarte et al., 1990), and the supraspinal sites, which involve activation of two distinct pathways, namely, the kyotorphin-met-enkephalin and NO-cGMP pathways (Kawabata et al., 1993). In addition to this claim, Kawabata et al. (1993) also suggested that the dual role played by NO in nociceptive processing at the supraspinal level is attributed to the types of pathway activated, in which the activation of the kyotorphin-met-enkephalin pathway was described to lead to antinociception, while activation of the NO-cGMP pathway resulted in hyperalgesia. The more complex nociceptive mechanism suggested for peripheral NO action, which depends on its tissue level (Kawabata et al., 1994), was based on the fact that NO produced inhibitory (antinociceptive) and promotive (nociceptive) effects (Abacioglu et al., 2000).

A study carried out by Larson et al. (2000) demonstrated the involvement of NO in pain perception at many levels of nociceptive neural pathways. They reasoned the presence of NOS peripherally, particularly in the primary afferent neurons and dorsal root ganglia, and centrally, such as in several sensory structures of the brain stem and thalamus, as the key factor for that. In addition, Rosland et al. (1987) have suggested the involvement of a glutamatergic N-methyl-d-aspartate (NMDA)-receptor mediated pathway in the nociceptive reflexes as the pathway mediates NO synthesis. According to them, activation of the said pathway and subsequent synthesis of NO will lead to enhancement of processing, or spinal facilitation of the afferent input that is ultimately conveyed to the cortex and subsequently manifested as behavioral responses.

In the current study, AECO was found to show concentration-independent antinociception with total loss of activity observed at the highest concentration (100%) used, which is in line with our recent report (Zakaria et al., 2005). It is believed that 25% AECO contained the bioactive compound responsible for activity, but in a quantity that is not high enough to produce significant antinociception, while 100% AECO exhibited a condition as described by Tripathi (2001) in which a drug’s effectiveness can sometimes be reduced due to the presence of high concentrations of active principles. They reasoned that in order to exert maximum curative effect, the concentrations of certain drugs used have to be within their respective therapeutic windows. On the other hand, Katzung (1995) suggested that deactivation of receptors, particularly the one involved in antinociception, due to presence of high concentration of active principles of drugs/extracts will lead to the loss of therapeutic activity of the respective drug/extract can also be used to explain the concentration-independent activity seen with AECO.

L-Arginine alone was found to induce hyperalgesia after systemic (s.c.) administration, which agrees with the report by Abacioglu et al. (2000). In addition, L-arginine, which is a precursor of NO production, was also found to reverse ASA, a nonsteroidal anti-inflammatory drug (NSAID), antinociception and, thus, supported the finding made by Bjorkman et al. (1994). According to Liying et al. (1997), NO, produced as a result of L-arginine breakdown by NO synthase, acts directly on the cyclooxygenase (COX) system, which may account for the loss of ASA antinociceptive activity. Interestingly, the current study demonstrated the ability of L-arginine to enhance 50% AECO antinociception while improving the ineffective 25% and 100% AECO activity. Based on our earlier discussion, it is plausible to suggest that the ineffectiveness of 25% AECO is due to the presence of lower amounts of active principles, while the ineffectiveness of 100% AECO is due to the phenomenon described by Tripathi (2001) or Katzung (1995), as mentioned above, and the ability of L-arginine to improve AECO antinociception might be attributed to the presence of L-arginine itself and not its by-product, NO. The reason for the latter suggestion is based on the fact that not the nociceptive effect elicited by L-arginine may represent direct actions on nociceptors (Kawabata et al., 1994) and would not be influenced by NO production.

L-NAME (20 mg/kg), an inhibitor of NOS, failed to affect the nociceptive threshold when given alone as reported by Jain and Kulkarni (1999). However, this contradicts the report made by Abacioglu et al. (2000)
that L-NAME antinociceptive activity falls within the range of between 18.75 and 150 mg/kg. This finding indicates that the decrease in endogenous NO within the peripheral tissue did not influence the algesia induced by acetic acid. In addition, L-NAME was also found to produce no significant effect on ASA antinociception, which refutes the report made by Bujalska and Gumulka (2001) who reported that NOS inhibitor like L\textsuperscript{G}-nitro-L-arginine (L-NO-ARG) increased the acetaminophen antinociception. Our finding was also in line with the report made by Talarek and Fidecka (2002) on the ability of L-NAME to enhance other antinociceptive agents (diazepam, chlordiazepoxide, and clonazepam) and, in this case, ASA and AECO. According to Ing et al. (1999), the ultimate effects of NO are dependent on dosage levels used and the rate and timing of NO release and could be used to explain our finding. Speculatively, it is suggested that the L-NAME significantly suppressed the NO concentration until there was not enough NO to directly activate the COX system (Liying et al., 1997), which is useful in explaining the well-maintained antinociceptive activity of ASA even after pretreatment with L-NAME. In terms of the AECO, L-NAME was found to enhance 50% AECO antinociception while significantly reversing the ineffective effect of 10% and 100% AECO. The ability of L-NAME to reverse both ineffective concentrations (10% and 100%) of AECO was not in agreement with the report by Jain et al. (2001) who indicated the ability of L-NAME to reverse sildenafil antinociception. Either AECO antinociceptive activity improved or intensified significantly under the presence of low concentration of NO, and this requires further research at the tissue level, which was not the objective of this study. What we can say is that inhibition of NO synthesis does influence AECO antinociception significantly. In addition, the ability of L-NAME to enhance low and high doses of AECO antinociceptive activity, which are ineffective when given alone, might also be attributed to the ability of the former to increase vascular permeability induced by acetic acid. L-NAME has been reported earlier to produce such effects that may lead to the observed antinociceptive activity, especially in chemically mediated nociception models (Kawabata et al., 1994).

MB, a simple inhibitor of guanylate cyclase (GC), has been widely used in research involving pain perception (Meller & Gebhart, 1993). GC is known to be affected by the presence of NO and, thus, MB is often used in the study of pain mechanisms involving the NO/cGMP pathway. Our study has demonstrated that MB produced remarkable antinociception when given alone, as reported by Abacioglu et al. (2000) and Talarek and Fidecka (2002), which seems to suggest an increase in pain threshold, possibly at peripheral and central levels, through deactivation of the guanylate cyclase. The highly significant intensification of 50% AECO antinociceptive activity by MB seems to indicate an important role of the guanylate cyclase and cGMP pathway in the observed activity of AECO. In addition, the ability of MB to reverse the ineffective concentrations (10% and 100% concentrations) of AECO into effective antinociceptive concentrations suggested that, at least, inhibition of the cGMP pathway is involved in the antinociceptive mechanism of AECO. The possible involvement of peripheral (Ferreira et al., 1991) as well as central (Meller & Gebhart, 1993) cGMP pathways in AECO antinociceptive activity could be suggested because AECO has also been reported to produce central antinociceptive activity, in addition to the peripheral one, when given systemically (s.c.) (Zakaria et al., 2005). Furthermore, morphine administered systemically has also been reported to produce central antinociceptive activity in addition to the peripheral activity, an activity that is also observed with AECO. Our finding on the involvement of opioid receptor in AECO antinociceptive activity might also help strengthen the above statement (Zakaria et al., 2005).

It is known that, depending on the biological system, cGMP modulates ion channels directly (Greger & Windhorst, 1996) or indirectly (via PKG stimulation and opening of K\textsuperscript{+}\textsubscript{ATP}) (Han et al., 2002). Several researchers (Rodrigues & Duarte, 2000; Alves & Duarte, 2002) have reported that part of the pharmacological event in the peripheral antinociceptive effect of morphine, dipyrone, sodium nitroprusside (NO donor), or dibutyryl cGMP assessed using a modified classical Randall-Sellito mechanical test resulted from the opening of K\textsuperscript{+}\textsubscript{ATP}. Thus, it was claimed that these peripheral analgesics block ongoing hypernociception by restoring the normal high receptor threshold via K\textsuperscript{+}\textsubscript{ATP} channel opening with a consequent increase in the K\textsuperscript{+} current. This channel is known to be opened either directly by cGMP or indirectly via protein kinase G (PKG) stimulation (Sachs et al., 2004). Because MB caused inhibition of cGMP production, it is plausible to suggest that the observed antinociceptive activity in AECO, other than being stimulated directly at the central level by the extract (Zakaria et al., 2005), was also caused by indirect opening of the K\textsuperscript{+}\textsubscript{ATP} channels via PKG stimulation (Sachs et al., 2004). However, investigation on the involvement of PKG in AECO antinociceptive activity is not part of the current study.

Nonetheless, it is worth highlighting that activation of GC in particular or the cGMP pathway in general has been reported to result in hyperalgesia rather than antinociception (Malmberg & Yaksh, 1993). On the contrary, some researchers considered cGMP and NO to work as mediators in pain perception. cGMP seems to play an important role in the functional up- and down-regulation of nociception. The discrepancy in results between the different studies may be explained on the basis of difference in pain models selected for analysis, the intensity and type of nociceptive stimulus employed, as
well as the animal species used (Deciga-Campos et al., 2004). Furthermore, the peripheral pronociceptive/antinociceptive activity of the NO-cGMP pathway may be affected by tissue levels of NO and/or the intracellular content of cGMP. So, changes in concentration of these mediators (e.g., by l-NAME and/or MB) may lead to contrasting effects (Kawabata et al., 1994).

The failure of l-NAME after pretreatment with l-arginine to change the latter effects, either alone or on ASA, seems to indicate the possibility of involvement of different nociceptive pathways that do not involve NO as a mediator in the observed activity of l-arginine. Speculatively, it can be suggested that l-arginine, other than being a precursor for the synthesis of NO, might also have a direct effect in the mechanisms of pain perception, and this requires further research. However, the involvement of NO could not be totally ruled out as it was an important modulator in the release and uptake of neurotransmitters known to be involved in the pain signal transmission (Katzung, 1995), like glutamate (Guevara-Guzman et al., 1994) and γ-aminobutyric acid (GABA) (Getting et al., 1996). Except for 100% AECO, pretreatment with l-NAME was found to cause no significant change in the effect of l-arginine on 10% and 50% AECO. Although there is an increase in the number of abdominal constrictions after pretreatment of 100% AECO with (l-NAME + l-arginine) when compared with the former, pretreated only with l-arginine, the result is only slightly significant.

The suppression of the l-arginine-induced nociception after coadministration with MB leads us to conclude that inhibition of guanylate cyclase, which then leads to deactivation of the cGMP pathway, will definitely cause antinociception, as reported by Jain et al. (2003). The presence of antinociceptive activity in the group pretreated with l-arginine and MB followed by ASA, or the three concentrations of AECO, seems to indicate that inhibition of the cGMP pathway even in the presence of NO synthesized from l-arginine will also lead to the observed antinociceptive activity. Speculatively, it is believed that l-arginine, but not NO, was responsible for the slightly significant decreased in the said activity. The suggested reason for this was that NO could not activate the cGMP pathway to produce nociceptive activity as it was already blocked by MB. However, the notion that l-arginine can also induce nociceptive activity requires further research, as mentioned earlier. Our observations showed that in the presence of l-arginine, the MB-enhanced antinociceptive effect of AECO is reduced to more than five-fold, as can be seen with 10%, 50%, and 100% AECO. The combined abilities of MB to inhibit l-arginine–induced NO and cGMP increases, which then caused vascular permeability in tissues, might explain the reversed, but retained antinociceptive activity of AECO (Abacioglu et al., 2000).

In the earlier part of the discussion, we have mentioned in greater detail that the l-arginine/NO/cGMP pathway, depending on the dose and site of administration, may have opposite effects, antinociception or nociception (Sousa & Prado, 2001). Based on these results, it was suggested that there are different subtypes of primary nociceptive neurons through which the l-arginine/NO/cGMP pathway causes contrary nociceptive effects (i.e., antinociceptive or hypernociceptive activities). Previous findings that activation of the l-arginine/NO/cGMP pathway promotes opposite modulation in the s.c. and intradermal tissue layers (Vivancos et al., 2003) can also be used to support our contrary finding to some of the previously reported observation in regard to l-arginine/NO/cGMP pathway.

Earlier studies on AECO antinociceptive activity have demonstrated the involvement of, at least in part, opioid receptor (Zakaria et al., 2005), and taking into account this recent study, our findings were in line with various reports that indicated the involvement of the l-arginine/NO/cGMP pathway in opioid-mediated antinociception (Duarte et al., 1990; Granados-Soto et al., 1997; Nozaki-Taguchi & Yamamoto, 1998; Abacioglu et al., 2000). Finally, we conclude that the AECO peripheral antinociception involved, at least partly, the l-arginine/NO/cGMP pathway.

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References


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