Effect of centrally administered nitric oxide modulators in Brewer’s yeast-induced nociception in rats

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Possible modulation of Brewer's yeast-induced nociception by centrally (icv) administered nitric oxide (NO) modulators, viz., NO synthase (NOS) inhibitors, NO precursor, donors, scavengers and co-administration of NO donor (SIN-I) with NOS inhibitor (L-NAME) and NO scavenger (Hb) was investigated in rats. Administration of NOS inhibitors and NO scavenger Hb increased the pain threshold capacity significantly, whereas NO donors SIN-I, SNP and NO precursor L-arginine were found to be hyperalgesic. D-arginine, the inactive isomer of L-arginine and methylene blue, inhibitor of soluble guanylate cyclase failed to alter the nociceptive behaviour in rats. Co-administration of SIN-I with L-NAME and Hb found to increase the nociceptive threshold. The results indicate, that centrally administered NO modulators alter the nociceptive transmission induced by Brewer's yeast in rats.

Nitric oxide (NO) is a free radical gas and is formed by NO synthase (NOS) on L-arginine. NOS is widely distributed in brain and peripheral tissue and exists in one of the three isoforms, two constitutive isoforms that are always present within the body (neuronal and endothelial NOS) and a third isoform that is not normally present and must be synthesized de novo (inducible NOS). Neuronal NOS (nNOS) and endothelial NOS (eNOS) are primarily, but not exclusively found within the nervous system, while inducible NOS (iNOS) is commonly found in a variety of cell types including macrophages, chondrocytes and neutrophils.

NO is a key mediator of nociceptive activity in numerous animal models used to study pain. Recent studies have suggested a role of NO, a second messenger in nociceptive reflexes as well as in modification of antinociception induced by morphine. L-arginine and NO donors such as sodium nitroprusside (SNP), nitroglycerin and 3-morpholinosydnonimine (SIN-I) have been demonstrated to exhibit antinociceptive activity in rats with carrageenin-induced hyperalgesia. In contrast, L-NAME suppresses, while L-arginine and NO donors enhance nociception or inflammatory response elicited by bradykinin, substance P, carrageenin and dextran. Thus, there exists evidence of both nociceptive and antinociceptive role of NO in peripheral tissues. Further, peripherally or centrally administered NOS inhibitors have been shown to produce antinociceptive effect in mice. NO found in the central nervous system plays an important role in the central processing of painful stimuli.

Although, there is considerable evidence implicating NO in carrageenin, formalin and bradykinin-induced nociception, there is no information on Brewer’s yeast-induced nociception. Hence, the present study has been undertaken to investigate icv administered isoform selective and non-selective inhibitors of NOS and other NO modulators in Brewer’s yeast-induced nociception in rats.

Materials and Methods
The studies were conducted on adult male Wistar rats (175-225 g). The animals (6 in each group) were housed individually with free access to clean drinking water and balanced feed. Experiments were conducted at an ambient temperature of 25±2°C between 0900 and 1600 hrs. ICV cannulation was performed stereotaxically by implanting a polyethylene cannula (No. 47) into the right lateral ventricles under ketamine hydrochloride (100 mg/kg, im) anaesthesia. The operated rats were allowed to recover for 7 days before conducting experiments. Drugs administered icv in rats included N-Nitro-L-arginine.
arginine methyl ester (L-NAME), L-N⁶-monomethyl arginine (L-NMMA), 7-nitroindazole (7-NI) and L-N⁶-iminoethyl lysine (L-NIL) and its inactive isomer D-arginine (50 µg each), 3-morpholinosydnonimine (SIN-1) and sodium nitroprusside (SNP) (500 and 250 µg, respectively), bovine hemoglobin (200 µg) and methylene blue (20 µg). Co-administration of SIN-1 (500 µg) with L-NAME (100 µg) and Hb (200 µg) was also done. Rats were administered drugs by icv 30 min prior to induction of nociception by Brewer’s yeast. All the drugs obtained from Sigma Chemical Co (U.S.A.) were dissolved in artificial CSF except for 7-NI which was dissolved in arachis oil. A constant volume of 5 µl of drug solution was injected icv using a 10 µl micro syringe. Control animals received equivalent volume of artificial CSF/arachis oil.

Rats received an intraplantar injection of 0.1 ml of 20% (W/V) Brewer’s yeast in normal saline solution in the right hind paw. The pressure in the Brewer’s yeast injected paw was recorded as pain threshold by Randall-Selitto assay immediately prior to and at 1, 3 and 5 hr post-Brewer’s yeast injection. In brief, pain threshold was measured by applying pressure to inflamed paw at steadily increasing rate by means of pedal switch of Randall-Selitto apparatus (UGO Basile, Italy). The end point or “Pain threshold” is defined as the pressure necessary to cause animals to struggle. The change in pain threshold in test groups was compared with that of untreated control group.

After termination of experiments, all the rats were administered 5 µl of 1% Evan’s blue dye solution icv and the brain was removed, sectioned and examined to ascertain the correct position of the cannula in the ventricles.

The results are presented as Mean ± S.E. Statistical analysis of the data was performed using Student’s t test to study the difference amongst the means.

### Results

The results of icv administered NO modulators in Brewer’s yeast-induced nociception in rats are summarized in Tables 1 and 2. The pain threshold increased significantly following icv administration of NOS inhibitors (Table 1) and bovine hemoglobin (Table 2), whereas l-arginine (Table 1), SIN-1 and SNP (Table 2) were found to be hyperalgesic. D-arginine and methylene blue failed to alter nociceptive responses in the present study.

The non-selective cNOS/iNOS inhibitors L-NAME and L-NMMA increased pain threshold capacity at 1 hr post-Brewer’s yeast injection up to 5 hr of observation period. Between the two non-selective NOS inhibitors, L-NMMA was found to be more effective antinociceptive as compared to L-NAME. The iNOS inhibitor 7-NI showed its antinociceptive effect starting at 3 hr and iNOS inhibitor L-NIL was found to increase pain threshold, starting at 1 hr and continued up to 5 hr observation period.

The NO precursor, L-arginine produced hyperalgesia from 3 hr onwards but its isomer D-arginine was without any effect. The NO donors, SIN-1 and SNP reduced the pain threshold significantly from 3 hr post Brewer’s yeast administration. NO scavenger hemoglobin showed antinociceptive effect starting at 3 hr to 5 hr, whereas soluble guanlyate cyclase inhibitor methylene blue failed to alter nociceptive response.

<table>
<thead>
<tr>
<th>NO modulator</th>
<th>Pain threshold (g)</th>
<th>0 hr</th>
<th>1 hr</th>
<th>3 hr</th>
<th>5 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>107.50 ± 5.60</td>
<td>85.83 ± 6.11</td>
<td>68.33 ± 6.79</td>
<td>60.00 ± 4.83</td>
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</tr>
<tr>
<td>L-NAME</td>
<td>92.50 ± 7.16</td>
<td>107.50 ± 7.72*</td>
<td>104.17 ± 9.52*</td>
<td>100.00 ± 7.64**</td>
<td></td>
</tr>
<tr>
<td>NMMA</td>
<td>105.00 ± 8.37</td>
<td>130.00 ± 9.21**</td>
<td>125.00 ± 9.04***</td>
<td>112.50 ± 9.64***</td>
<td></td>
</tr>
<tr>
<td>7-NI</td>
<td>108.33 ± 7.15</td>
<td>98.33 ± 8.91</td>
<td>103.33 ± 6.54**</td>
<td>103.33 ± 6.67**</td>
<td></td>
</tr>
<tr>
<td>L-NIL</td>
<td>108.33 ± 8.63</td>
<td>143.33 ± 8.43***</td>
<td>135.00 ± 7.30***</td>
<td>129.17 ± 8.80***</td>
<td></td>
</tr>
<tr>
<td>NO precursor</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>81.67 ± 7.26</td>
<td>75.83 ± 6.64</td>
<td>66.67 ± 6.67</td>
<td>61.67 ± 6.01</td>
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<tr>
<td>L-arginine</td>
<td>86.67 ± 8.82</td>
<td>71.67 ± 10.38</td>
<td>50.00 ± 3.65*</td>
<td>42.50 ± 5.44*</td>
<td></td>
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<tr>
<td>D-arginine</td>
<td>86.67 ± 10.85</td>
<td>77.50 ± 7.27</td>
<td>74.17 ± 6.38</td>
<td>64.17 ± 5.69</td>
<td></td>
</tr>
</tbody>
</table>

P values: *<0.05; **<0.01; ***<0.001

Table 1—Effect of centrally administered NOS inhibitors and NO precursor on Brewer’s yeast-induced nociception in rats

[Values are mean ± S. E. from 6 animals in each group]
Co-administration of SIN-1 with L-NAME reduced pain threshold as compared to control, whereas administration of SIN-1 and Hb increased pain threshold significantly.

**Discussion**

The choice of yeast as the irritant was based on the production of a large oedematous response and concomitant development of strong hyperalgesia. Nociceptive stimuli may involve production of NO, since isoforms, non-selective and selective NOS inhibitors (L-NAME, L-NMMA, 7-NI and L-NIL) and bovine hemoglobin (Hb), scavenger of NO were found to increase pain threshold significantly. The isoform non-selective NO inhibitors (L-NAME and L-NMMA), nNOS inhibitor (7-NI) and iNOS inhibitor (L-NIL) when given icv 30 min prior to induction of algiesia increased the pain threshold indicating the involvement of endogenous NO in nociceptive process. Depending upon the time course, NO may be generated either by cNOS and/or iNOS or by both the isoforms.

The precise site of action of the antinociceptive effect of L-NAME remains unclear but icv administered L-NAME reduced formalin-induced paw licking time, strongly suggesting an effect within the central nervous system. Furthermore, L-NAME is antinociceptive in hot-plate assay, which is widely believed to be sensitive solely to drugs acting supraspinally. Moore and co-workers reported that ip injections of the nNOS inhibitor 7NI, significantly reduced hind-paw licking behaviour due to formalin. Intrathecal (it) administration of iNOS selective inhibitor aminoguanidine was able to inhibit thermal, but not mechanical hyperalgesia in zymogen inflammation model. Similar results were reported in rats pretreated with another selective iNOS inhibitor L-NIL. 7-NI like L-NAME inhibits late phase formalin-induced hind paw licking by virtue of decreasing the formation of NO in the dorsal horn of spinal cord.

L-arginine but not D-arginine caused enhancement of Brewer's yeast-induced nociception. ICV administered l-arginine elicited antinociception in mice as assessed by the tail-flick test. The antinociceptive effect of L-arginine was attributed to the formation of kytoporin (L-Tyr- L-Arg) in the brain. Chronic administration of L-arginine causes selective increase (15%) in the NOS activity in the mid brain. The mid brain is an important region involved in pain perception and contains periaqueductal grey matter which has high density of endogenous opioid receptors. The increase in NOS activity would enhance the production of NO, which appears to have an algies effect. The enhancement of nociceptive behaviour by L-arginine suggests its role as a substrate for NOS.

The NO donors SIN-1 and SNP-induced hyperalgesia in rats demonstrate that endogenous NO released from NO donors is also involved in the nociceptive response in Brewer's yeast induced nociception. Similar findings were also reported in
tail-flick test in rats by intrathecally administered NO donors.\(^{30,31}\)

Hemoglobin, a scavenger of NO, increased the pain threshold significantly, whereas the nociceptive response was not modified by methylene blue showing that endogenous NO modulates Brewer's yeast-induced nociception by a cyclic GMP independent mechanism. Hemoglobin produces its effect by binding NO at the extracellular site.\(^{32}\)

Methylene blue failed to alter the tail-flick nociception when injected ivc alone and did not affect the baseline threshold of tail-flick response.\(^{33}\)

Co-administration of SIN-1 with L-NAME reduced pain threshold as compared to control group which further confirms the involvement of exogenous NO from SIN-1 in nociception as endogenous NO synthesis was blocked by NOS inhibitor L-NAME. Administration of Hb in combination of SIN-1 and Hb alone is effective in scavenging both exogenous and endogenous NO, respectively.

From the present investigation, it is concluded that central nitrinergic system modulates Brewer's yeast-induced nociception in rats. It was also observed that ivc administration of selective inhibitors of both nNOS and iNOS during time course of Brewer's yeast-induced inflammation significantly reduced nociception. It is concluded that increased induction of brain nNOS in late period of inflammatory response contributes to Brewer's yeast-induced nociception. On the other hand, brain iNOS most likely contributes to pain in both early and late period of the Brewer's yeast inflammatory response. Furthermore, nociception induced by NO donors was attenuated by Hb.

References
20. Meller S T & Gebhart G F. Nitric oxide (NO) and nociceptive processing in the spinal cord, Pain, 52 (1993) 127.


