Effect of VMH Lesion on Sucrose-Fed Analgesia in Formalin Pain

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Abstract: The ingestion of sucrose (ad libitum) produces an immediate analgesic response to phasic noxious stimuli. The underlying mechanism for the analgesic effect of sucrose is attributed to its palatability, which mediates analgesia probably by the release of β-endorphin in the hypothalamus. The present study was designed to explore the role of ventromedial hypothalamus in the mediation of sucrose-fed analgesia. Adult male albino rats each received (20%) sucrose solution orally through a separate bottle until they had ingested 4–5 ml. Their behavioral responses to tonic noxious stimulus in a formalin test were studied in pre- and postsucrose-fed rats of control and in the VMH lesion groups. The average pain rating of a 60-min session significantly \( (p<0.01) \) decreased after sucrose feeding in control rats, from 1.94±0.13 to 1.45±0.14, but sucrose feeding by the VMH lesion rats did not alter their tonic nociceptive response from a 1.70±0.07 presucrose-fed state to a 1.71±0.08 postsucrose-fed state. VMH lesion per se did not alter the nociceptive response in comparison with controls. The results suggest that sucrose feeding produces analgesia to tonic noxious stimulus, which is abolished by lesion of the VMH, thereby indicating a significant role of VMH in sucrose-fed analgesia. [Japanese Journal of Physiology, 51, 63–69, 2001]

Key words: sucrose-fed analgesia, formalin pain, tonic pain, VMH.

The ingestion of sucrose has been reported to produce analgesia in newly born rat [1], human [2, 3], and adult rat models [4]. Sucrose ingestion in rat pups [1] immediately increases paw lift latency to thermal noxious stimulus, and in adult rats it decreases nociceptive behavioral rating to tonic formalin stimulus after 12 h [5]. In newborn infants, it reduces crying time, attenuates the increase in heart rate and blood pressure, and improves oxygen saturation, indicating analgesia [2, 3, 6]. This analgesic effect is clinically utilized to carry out minor surgical interventions such as immunization, circumcision, and venesection in newborn infants with no other anesthetic supplementation [2, 3, 6, 7]. The analgesic action is possibly not secondary to distraction, but related to the hedonic qualities of sucrose [8]. The ingestion of palatable sweet substances such as candy is reported to decrease the levels of hypothalamic β-endorphin and opioid receptor binding probably because of increased utilization [9]. Moreover, the involvement of opioid peptide is further supported by abolition of the sucrose-fed analgesia in naloxone pretreated infants [2].

Medial hypothalamus is involved in the modulation of pain, since its lesion produces hyperalgesia [10, 11] and stimulation produces analgesia [12, 13]; its neurons have opioid receptors [14–16]. These neurons also respond to iontophoretic application of glucose. These glucose-responsive neurons respond to both the peripheral noxious stimulation and micro iontophoretic application of opioid peptides by attenuating their firing rate [14–16]. There are therefore indirect indications of a link between opioid, analgesia, and food ingestion. It is not known whether the sucrose-fed analgesia is also neurally mediated. Similarly, the VMH also governs the preference for palatable food and is also influenced by palatable food [9]. The VMH therefore exerts a significant effect in pain modulation and carbohydrate preference, although its
role in pain modulation by palatable food has not been reported. The present study was therefore aimed to explore the role of the VMH, if any, in sucrose-fed analgesia.

MATERIALS AND METHODS

Adult male wistar rats of 150 to 200 g were housed individually in polypropylene cages where 26±2°C temperature and a 14:10 h light/dark cycle were maintained. They were provided laboratory food pellets and tap water ad libitum. Their food and water intake were noted daily and their body weight weekly.

On the day of experiment, the rats \( n=6 \) underwent tests for response to tonic noxious stimulus in 1-h sessions (presucrose-fed state, control group). The experiments were conducted in strict compliance with the International Association for the Study of Pain [17]. A separate group of rats \( n=6 \) had access to freshly prepared 20% sucrose solution per oral route through an additional graduated container attached with a stopper and a nozzle (sucrose-fed group). The rats ingested 4 to 5 ml of it during 5 to 7 min after the first lick. The sucrose was then withdrawn and the algogen was injected to study the effect of sucrose feeding on tonic nociceptive behaviour.

A separate group \( n=6 \) of age/sex–matched rats received lesions of VMH. In the anesthetized (ketamine hydrochloride: KETAMIN-50, I.M. 50 mg/kg body weight) rats DC current (2 mA for 15 s) was passed through a bipolar electrode implanted in VMH by using 2.8 mm AP, 0.5 mm lateral, and 9.5 mm vertical coordinates from the Paxinos and Watson rat brain atlas [18]. After surgical recovery (2 weeks), response to tonic noxious stimulus was noted (lesion group), which was repeated after 1 week in the sucrose-fed state (lesion–sucrose-fed group).

The rats were tested for response to tonic noxious stimulation by injecting algogen (sterile formalin 5%, 50 µl) subcutaneously into the plantar surface of the forepaw [19]. Their pain response was evaluated by more than one experimenter using the “blind” method. The pain-related behavior was recorded by using a 4-point scale: category 0, when the whole body of the rat was resting or moving while the body weight was equally borne by all the paws; category 1, grooming or the partial protection of the injected paw by not bearing the body weight on that paw; category 2, the injected paw was kept elevated or tucked into the body; category 3, the injected paw was licked or shaken. These categories were continuously fed into a personal computer for an hour. The time spent \( (T) \) in each category, namely 0 through 3 \( (T0, T1, T2, T3) \) in blocks of 5 min, the average pain rating during each block and of the 1-h session were obtained through a software program described elsewhere [20]. Briefly, pain rating was calculated as the sum of \( T1 + 2T2 + 3T3 \) divided by the duration \( (t, s) \).

At the end of the experiment, the animals were sacrificed under deep anesthesia by an overdose of ether and perfused intracardially with 10% formalin. The brain was removed and processed for histological verification of the lesion site.

Data analysis. The data was analyzed by applying the Kruskal Wallis Test for evaluating the overall significance among the control, sucrose-fed, and VMH lesion group of rats. The pain rating of all blocks and the duration of all four categories were compared. Wherever, the data attained significance that was further analyzed by the Wilcoxon Ranksum test. The data of the VMH lesion and lesion–sucrose-fed groups were compared by using a paired \( t \)-test.

RESULTS

The pain rating during the 60-min period of observation is presented in 12 blocks of 5 min each in the control rats (Table 1). It attained a peak value of \( 1.79±0.58 \) in the I block, then decreased to \( 1.42±0.58 \) in the II block. During the III block, however, the rating started increasing again and reached a second peak \( (2.16±0.36) \) during the V block. The pain then persisted until the 60-min period of observation.

Immediately following a formalin injection, the rat spent \( 89.89±67.02 \text{s} \) in category 3 (Fig. 1), \( 100.81±25.10 \text{s} \) in category 2 (Table 2, Fig. 1), and \( 65.99±54.59 \text{s} \) in category 1 during the I block (Table 3, Fig. 6).

Table 1. Effect of sucrose feeding to control rats on the average pain rating.

<table>
<thead>
<tr>
<th>Block</th>
<th>Time after sucrose feeding (min)</th>
<th>Average tonic pain rating (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>Sucrose-fed group</td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>1.79±0.58</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>1.42±0.58</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>1.84±0.58</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>2.04±0.29</td>
</tr>
<tr>
<td>V</td>
<td>25</td>
<td>2.16±0.36</td>
</tr>
<tr>
<td>VI</td>
<td>30</td>
<td>2.11±0.21</td>
</tr>
<tr>
<td>VII</td>
<td>35</td>
<td>2.08±0.37</td>
</tr>
<tr>
<td>VIII</td>
<td>40</td>
<td>1.99±0.21</td>
</tr>
<tr>
<td>IX</td>
<td>45</td>
<td>2.07±0.12</td>
</tr>
<tr>
<td>X</td>
<td>50</td>
<td>1.97±0.08</td>
</tr>
<tr>
<td>XI</td>
<td>55</td>
<td>1.95±0.25</td>
</tr>
<tr>
<td>XII</td>
<td>60</td>
<td>1.86±0.24</td>
</tr>
</tbody>
</table>
1. The rat gradually spent more time in category 2, from 100.81 ± 25.10 s in the I block to 271.18 ± 37.17 s in the X block (Table 2).

After ingestion of sucrose, the average pain ratings during the I block (1.73 ± 0.27) and through the III block (1.82 ± 0.43) did not statistically differ significantly from that of the control (1.79 ± 0.58 in the I block and 1.84 ± 0.58 in the III block) group of rats (Table 1). The pain rating decreased more during the IV–VI and VIII–IX blocks (p < 0.05 and p < 0.01, respectively) than in the control group of rats. The rats spent less time in categories 2 and 3 and more time in category 1 (Tables 2 and 3, Fig. 1).

The lesion site was confirmed histologically in the VMH (Fig. 2). The daily food intake of the control rats ranged from 23.23 ± 4.32 to 25.66 ± 4.30 g throughout the period of observation, and that of the lesion group of rats increased to 28.60 ± 2.80 g (2nd postlesions day) and 33.30 ± 3.86 g (14th postlesion day) (Table 4). It continued to remain significantly higher statistically than their prelesion values until the period of observation ended. The body weight of the lesion group of rats increased (p < 0.01) to 196.50 ± 13.93 g, from 166.80 ± 9.25 g, during the 4-week period of observation.

The pattern of nociceptive behavioral rating in the lesion group was similar to that of the control rats. The initial peak in pain rating was reached during the I block (2.04 ± 0.3) and the second peak during the VI block (1.83 ± 0.33) (Fig. 3).

When the time spent in each category of formalin pain between the lesion and control groups of rats is compared, there was no difference in the duration of category 3. The duration of category 2 was statistically less and the duration of category 1 much higher in lesion in comparison with the control rats (Fig. 4).

Table 2. Effect of sucrose feeding to control and sucrose-fed rats on category 2 of formalin pain.

<table>
<thead>
<tr>
<th>Block</th>
<th>Time after sucrose feeding (min)</th>
<th>Duration in category 2 (mean ± SD, s)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>100.81 ± 25.10</td>
<td>0.023</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>135.70 ± 69.69</td>
<td>0.046</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>137.90 ± 95.15</td>
<td>0.086</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>191.47 ± 64.22</td>
<td>0.047</td>
</tr>
<tr>
<td>V</td>
<td>25</td>
<td>155.23 ± 72.77</td>
<td>0.009</td>
</tr>
<tr>
<td>VI</td>
<td>30</td>
<td>176.03 ± 92.32</td>
<td>0.307</td>
</tr>
<tr>
<td>VII</td>
<td>35</td>
<td>218.24 ± 73.89</td>
<td>0.085</td>
</tr>
<tr>
<td>VIII</td>
<td>40</td>
<td>257.91 ± 51.35</td>
<td>0.008</td>
</tr>
<tr>
<td>IX</td>
<td>45</td>
<td>251.59 ± 34.01</td>
<td>0.568</td>
</tr>
<tr>
<td>X</td>
<td>50</td>
<td>271.18 ± 37.17</td>
<td>0.208</td>
</tr>
<tr>
<td>XI</td>
<td>55</td>
<td>244.39 ± 80.90</td>
<td>0.413</td>
</tr>
<tr>
<td>XII</td>
<td>60</td>
<td>241.26 ± 81.76</td>
<td>0.130</td>
</tr>
</tbody>
</table>

Table 3. Effect of sucrose feeding to control rats on category 1 of formalin pain.

<table>
<thead>
<tr>
<th>Block</th>
<th>Time after sucrose feeding (min)</th>
<th>Duration in category 1 (mean ± SD, s)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>65.99 ± 54.59</td>
<td>0.003</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>47.49 ± 44.32</td>
<td>0.005</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>75.38 ± 70.23</td>
<td>0.040</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>41.90 ± 65.26</td>
<td>0.011</td>
</tr>
<tr>
<td>V</td>
<td>25</td>
<td>37.10 ± 49.71</td>
<td>0.001</td>
</tr>
<tr>
<td>VI</td>
<td>30</td>
<td>43.44 ± 48.70</td>
<td>0.026</td>
</tr>
<tr>
<td>VII</td>
<td>35</td>
<td>28.24 ± 28.34</td>
<td>0.020</td>
</tr>
<tr>
<td>VIII</td>
<td>40</td>
<td>2.89 ± 7.07</td>
<td>0.004</td>
</tr>
<tr>
<td>IX</td>
<td>45</td>
<td>8.83 ± 12.98</td>
<td>0.059</td>
</tr>
<tr>
<td>X</td>
<td>50</td>
<td>18.31 ± 28.39</td>
<td>0.324</td>
</tr>
<tr>
<td>XI</td>
<td>55</td>
<td>31.19 ± 65.65</td>
<td>0.274</td>
</tr>
<tr>
<td>XII</td>
<td>60</td>
<td>47.46 ± 74.99</td>
<td>0.173</td>
</tr>
</tbody>
</table>
Sucrose feeding by the VMH lesion rats did not affect
the pattern of nociceptive behavior, the average pain
rating, or the duration spent in each category in com-
parison with their pain rating in lesion per se state
(Figs. 3, 4).

**DISCUSSION**

Short-term sucrose feeding in our study decreased the
pain rating, thereby indicating analgesia, and the
VMH lesion abolished sucrose-fed analgesia. The re-
results suggest that sucrose feeding relieves tonic pain
and that the VMH mediates sucrose-fed analgesia.

Our results of relief in pain by sucrose ingestion are
similar to those reported by several workers in the past
[21, 22]. However, they have reported a relief for the
phasic pain such as thermal noxious stimulus. An in-
fusion of 7.5% sucrose solution in a 10-d-old rat pup
significantly elevated paw lick latency in comparison
with water-infused controls, though the analgesia was
not sustained. Sucrose has been given to newborn in-
fants also for such procedures as immunization, heel
lance, and circumcision [2, 22, 23], thereby, suggest-
ing that the effect may last for a variable period of 0 to
20 min. In a formalin model, the noxious stimulus
produces an initial greater pain in response to direct
stimulation of the nociceptors, which lasts for a
shorter duration, whereas the late pain in this model
that is sustained for 3 to 4 h is due to inflammation
and prolonged neural changes initiated by early phase
activity. An ingestion of 4 to 5 ml of sucrose by our
rats in a short span of 5 to 7 min statistically reduced
the pain significantly in the late phase (45 min), but
not in the initial phase. The longer duration of effect is
probably because of a higher strength of sucrose solu-
tion in our rats. Blass et al. [8, 21] have suggested,
however, that the strength of a sucrose solution does
not influence the response to noxious stimulus. Our
rats were adults and ingested 20% sucrose solution
while they were giving 3.5, 7.5, and 11.5% sucrose
solution to rat pups. It appears that the analgesic effect
is independent of sucrose solution strength, although

![Fig. 2. Depicts camera lucida drawings of brain sections corresponding to bregma −1.8, −2.8, −3.3, −3.8, showing the extent of VMH lesion.](image_url)
the duration of effect possibly appears to be dependent
on it.

In our rats, VMH lesion abolished the sucrose fed
analgesia probably because the ingestion of sucrose
and palatable food [9, 24]. These endorphin-rich neurons
have cell bodies in arcuate nucleus [24]. In our experi-
ments, the electrolytic lesion of the VMH leads to
neuronal and axonal fiber loss. The loss of endorphin-
ergic fibers misses the link in the neural circuitry of
the sucrose-fed analgesia and therefore leads to an
abolition of the analgesic effect of sucrose. VMH is
well connected with periaqueductal gray (PAG), both
directly and indirectly [25]. PAG activates the endoge-
nous analgesic system. Moreover, VMH has a direct
influence on nucleus raphae magnus (NRM) [26] and
the spinal cord [27]. It is also connected with the lim-
bic structures involved in tonic pain [28, 29]. Probably
it is through this route that the pain is reduced and the
lesion of the VMH abolished the effect.

Substantial evidence is provided in regard to the in-
volved VMH in tonic pain [30]. ϒ-Endorphin
immunoreactivity in the VMH increases following
formalin-induced tonic pain, thus implying activation
of the analgesic system to reduce the pain. Formalin
injection itself or the initial pain because of it acti-
vates the analgesic system. Sucrose feeding also pro-
duces analgesia probably as a result of the release of
ϒ-endorphin from the arcuate nucleus. Therefore the
analgesic effect of sucrose ingestion was marked,
which was evident in the late phase of formalin pain only. It lasted until the period of observation (60 min) ended, although the values were statistically significant for 45 min. The b-endorphin release is probably greater than that by the individual stimulus of sucrose ingestion or formalin injection.

In comparison with our previous report, the lesion of the VMH in this study unexpectedly did not lead to hyperalgesia when the average pain rating was compared with the control group [5]. A careful examination of our data revealed that the time of the day influences the hyperalgesic response. In our lesion rats, hyperalgesia to tonic noxious stimulus was noted during daytime hours (11:00), but in the late evening hours (17:00) when these experiments were conducted the rats remained eualgesic [5]. Further experiments are in progress to decipher the underlying reasons. This may possibly be due to the shift and variation in the influence exerted by different neural areas corresponding to the hour of the day on tonic pain response or the cyclicity of the endogenous analgesic system.

The results suggest that VMH exerts a critical influence on sucrose-fed nociceptive response to tonic pain. The VMH lesion did not alter the tonic pain response from its baseline value; however, it abolished the sucrose-fed analgesia in these rats.

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