

# How lidocaine acts in morphine dependency

Behnaz Sedighi, MD, Abbas Haghparast, PhD, Tajpary Klantary, MSc, Maryam Taieban, MD.

## ABSTRACT

**Objective:** To study the effect of analgesia caused by a local anesthetic agent (Lidocaine) in morphine dependent and independent rats.

**Methods:** We carried out this experimental study in the Neuroscience Research Center of Kerman Medical University, Iran in 2003. We evaluated 2 groups of morphine dependent and independent rats. Morphine dependency was induced orally, and formalin was used as a noxious stimulus. The orofacial formalin test in rats is a valid and reliable model of nociception. The formalin test induces 2 distinct periods of nociception reaction, the first phase occurs in the first 3 minutes and the second phase 15-45 minutes later. The behavioral response of the animals to the noxious stimulus (formalin) was measured by the time the animal spent rubbing the injected area. All the injections were carried out subcutaneously into the upper lip of the animal, at the same site if possible. The effect of morphine dependency on local analgesia was assessed by injection

of 50  $\mu$ L lidocaine prior to 50  $\mu$ L diluted formalin (2.5% in saline) in one group, and after formalin in the other group.

**Results:** Subcutaneous injection of lidocaine prior to morphine completely abolished the first phase of formalin nociceptive response in both morphine dependent and independent rats. Injection of lidocaine after formalin did not affect the first phase in both groups, but abolished the first part of the second phase in both groups.

**Conclusion:** Considering different mechanisms of morphine and lidocaine in the body, the results verified that the analgesia induced by lidocaine in both morphine dependent and independent groups was the same, and we do not need higher doses of lidocaine to suppress formalin induced pain in the morphine dependent group.

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Pain has a dual nature, from one aspect it is caused by a specific stimulus, but the most abstruse problem is pain quality that is due to one's psychiatric characteristics,<sup>1</sup> so is specific for each individual. Pain control is one of the main aims of human beings, and various substances by different mechanisms have been tested, and there are different ideas about pain control in morphine dependent persons.<sup>2,3</sup> All opioid drugs cause analgesia, and have all the effects of endogenous opioids (endorphins) on 3 main receptors ( $\mu$ ,  $\kappa$ ,  $\delta$ ) in the spinal cord, brain stem and peripheral tissues. They also have pre and postsynaptic effects on the posterior horn of the spinal cord and suppress the afferent pain impulses in C and A -  $\delta$  fibers. Opioids have at least 3 main receptors,<sup>4,5</sup> and via

these receptors, affect passage of ions, replacing intracellular  $\text{Ca}^{2+}$  and phosphorylation of proteins. They have 2 main proven effects. One is to close the voltage dependent  $\text{Ca}^{2+}$  channel on presynaptic neural terminals with decreased release of neurotransmitters (glutamate, norepinephrine, acetylcholine and substance - p) from afferent fibers, and the other effect is hyperpolarization and suppression of post synaptic neurons through the opening of  $\text{K}^+$  channels.<sup>5</sup> Connection of opioids to peripheral receptors mainly affects the inflammatory related pain.<sup>6</sup> There are different theories about the effect of local anesthetic agents, the most important of which is the presence of specific receptors theory.<sup>7</sup> According to this theory, local anesthetic agents connect to  $\text{Na}^+$  channels so that

From the Department of Neurology (Sedighi, Taieban), Shafa Hospital, Department of Physiology & Pharmacology (Haghparast), Faculty of Medicine, Kerman University of Medical Sciences, and the Faculty of Nursing (Klantary), Kerman Azad University, Kerman, Iran.

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Address correspondence and reprint request to: Dr. Behnaz Sedighi, Neurology Ward, Shafa Hospital, Kerman, Iran. E-mail: be\_sedighi@yahoo.co.uk

they have a direct effect on specific receptors, but do not affect the specificity of the neural membrane. It is proven that the primary effect of local anesthetic agents is on the depolarization phase of action potential. These effects cause a decrease of the depolarization in the slow phase, so the induced depolarization is not enough to reach the threshold of stimulation, but do not make changes in the repolarization phase. They decrease permeability of channels to  $\text{Na}^+$  and decrease passage of  $\text{K}^+$  from neural membranes. The  $\text{Ca}^{2+}$  that are banded to the internal surface of neural membrane regulate the passage of  $\text{Na}^+$ . Releasing the banded  $\text{Ca}^{2+}$  from membrane receptors may be the primary factor responsible for decreasing permeability of neural membrane to  $\text{Na}^+$  as the first step of depolarization. These drugs can act as a competitive  $\text{Ca}^{2+}$  antagonist in some parts of the neural membrane.<sup>7</sup> The formalin test has been used as a model of noxious stimulation in animals.<sup>8</sup> Subcutaneous (sc) injection of diluted formalin provides a sustained noxious stimulus and induces a diffuse long lasting pain that mimics some features of post injury pain in human beings. After injection of formalin, the nociceptive behavior consists of an early short lasting behavior (first phase) followed by a late prolonged response (second phase). Such biphasic responses to formalin have also been observed during electrophysiological recordings of convergent spinal dorsal horn, and trigeminal brain stem neurons.<sup>9</sup> Results of the study demonstrate that both the first and the second phases of the response to formalin are primarily due to peripheral inputs, consequently, the second phase can not be mediated by central sensitization alone.<sup>10</sup> Morphine dependency was caused orally by water containing morphine sulfate as the only fluid the animal drinks.<sup>11</sup> In this study, sc formalin was used to induce pain, and lidocaine was used to test the effect of a local anesthetic agent on opium dependent and independent rats.

**Methods.** The experimental study was carried out in the Neuroscience Research Center of Kerman Medical University, Iran in 2003. NMRI adult male rats weighing 150-200 gm were used in this study. The rats were housed in group cages (4 in each) with free access to food and water before the experiments. The morphine dependent group drank water containing morphine-sulfate. The rats were housed in a controlled climate and light situation ( $23 \pm 1^\circ\text{C}$ , 12 hours dark/light cycles with lights on at 07:00). Each animal was placed in the test box (30 cm x 30 cm x 50 cm glass box) before the experiments to decrease stress. Testing took place during the light phase between 07:00 and 14:00.

**Morphine dependency induction.** Due to the similarity to the addiction process in human beings, which is a chronic process, the oral method with morphine-sulfate was chosen.<sup>11</sup> To induce morphine

dependency, morphine-sulfate was added to water with the dosage of 0.1, 0.2, and 0.3 mg/ml, each dose for 48 hours, and then 0.4 mg/ml until 15 days. Following this, some of the rats received 3 mg/kg of Naloxone intraperitoneally and were observed for one hour to evaluate withdrawal signs.<sup>11,12</sup>

**Formalin test for orofacial pain.** In this experiment, the model of formalin test was sc injection of 50  $\mu\text{L}$  of diluted formalin (2.5% in saline) into the upper lip of the rat, just lateral to the nose.<sup>13</sup> To minimize injury, and unwanted reactions of the animals, a 1 ml syringe with a 0.4 x 35 mm needle (Gauge 27) was used for formalin delivery. The formalin solution was prepared from 37% stock formalin, which was diluted in 0.9% saline to obtain a 2.5% solution. Following injection, the rat was immediately returned to the glass box placed on transparent glass, on a mirror with the angle of  $45^\circ$ . The investigator observed the nociceptive behavior of the animal directly in the mirror. Each rat was observed for a period of 45 minutes, divided into 15 blocks of 3 minutes. Pain score was determined by measuring the number of seconds (amplitude) that the animal spent rubbing the injected area with its paw in each block.<sup>13</sup> All the animals were injected at the same site on the right side of the upper lip to observe the nociceptive response easily, furthermore, both injections in one rat tried to be in the same site if possible. The animals were used once only, and were then killed at the end of the experiment by a lethal dosage of pentobarbital to avoid unnecessary suffering.

**Morphine independent rats.** The group of morphine independent rats was divided into 6 subgroups. In each group, 8 rats were studied, except for the saline groups, which included 5. Group (A) - control group (n=8) received 50  $\mu\text{L}$  of normal saline (N/S) 0.9%, 5 minutes before the administration of 50  $\mu\text{L}$  of diluted formalin (2.5% in saline). It is important to note that, the amount of substance injected was 50  $\mu\text{L}$  in each injection. We also used 2% lidocaine without vasoconstrictor, diluted 2.5% in saline formalin and 0.9% N/S, for all experiments. Group (B) - lidocaine-formalin group (n=8) received lidocaine, 5 minutes before the administration of formalin. Group (C) - saline group (n=5) received 2 injections of N/S in 5 minute intervals. Group (D) - control group (n=8) received N/S 9 minutes after administration of formalin. Group (E) - formalin-lidocaine group (n=8) received lidocaine, 9 minutes after receiving formalin. Group F - saline group (n=5) received N/S 2 times in 9 minute intervals.

**Morphine dependent rats.** This group was divided into 7 subgroups, because 8 rats were chosen randomly as the naloxone group (group a), and received 3 mg/kg of naloxone intraperitoneally. These groups were observed for one hour to examine withdrawal signs. They showed at least 4 signs of withdrawal criterion,

and jumping was seen in all 8 rats, so the rats were dependent.<sup>12</sup> Group (b) - lidocaine – formalin group (n=8) received lidocaine, 5 minutes before the administration of formalin. Group (c) - saline group (n=5) received N/S twice at 5 minute intervals. Group (d) control group (n=8) received N/S 5 minutes before the administration of formalin. Group (e) - formalin – lidocaine group (n=8): received lidocaine 9 minutes after the administration of formalin. Group (f) saline group (n=5) received N/S twice at 9 minute intervals. Group (g) - control group (n=8) received N/S 9 minutes after administration of formalin.

Each rat in each subgroup was observed for 45 minutes. The animal reaction to the first phase of formalin stimulation (3 min) and the second phase (15 – 45 min later) were recorded as per Dallel's study<sup>10</sup> for comparison. The 45 minute period was divided into 15 blocks of 3 minutes, and in each block the seconds that the animal spent rubbing the injected area was recorded. The morphine dependent and independent groups were divided into control, saline, lidocaine – formalin and formalin – lidocaine subgroups and were compared with the rats in the same and opposite group.

**Statistical analysis.** Data are expressed as mean  $\pm$  SEM, and analyzed by analysis of variance (ANOVA). The data were subjected to one-way and two-way ANOVA and followed by protected Tukey's test for multiple comparisons, as needed. Alternatively, for comparison of the amplitude of rubbing in different intervals in one group, repeated measures model of ANOVA was used. A block design model of ANOVA was also used for comparing the amplitude of rubbing at different intervals (15 blocks of 3 minutes) in different groups of independent and dependent rats. For all tests, the level of significance was set at  $p < 0.05$ .

**Results. Morphine independent rats: Saline-saline group.** There was no significant difference in the severity of the animal behavioral reaction in the 2 saline groups (with 5 and 9 minute intervals).

**Saline-formalin and formalin-saline groups or control groups.** The rats that received 50  $\mu$ l of N/S, 5 minutes before or 9 minutes after the administration of formalin (50  $\mu$ l, 2.5%) showed a biphasic nociceptive response, the first phase was shorter (in the first 3 minutes), and the second phase was longer (15-45 minutes from injection). These 2 phases were separated with a period of no nociceptive response (from the 2nd to the 5th intervals) in this period. There was no significant difference between these 2 groups and the saline group.

**Lidocaine-formalin group.** Administration of lidocaine, 5 minutes before the sc injection of formalin, completely abolished the first phase of

animal nociceptive response and the animal response was the same as the saline group (in the first phase). In this phase, there was a significant difference, between this group and the control group ( $p < 0.001$ ). Furthermore, the sixth interval was also blocked, so that this suppression was one interval more than the control group. In other intervals, there was no significant difference between this group and the control group (**Figure 1**).

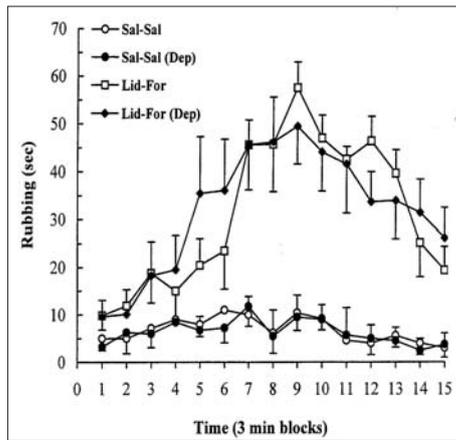
**Formalin-lidocaine group.** The first phase of the nociceptive response in this group was the same as the control group, but the second phase began after the 8th interval. From the second to the eighth intervals, there was no significant difference between this group and the saline group. Therefore, the sc injection of lidocaine has increased the phase of no nociception response for 3 intervals more than the control group. After the 8th interval, the nociceptive response was the same as the control group. The sc injection of lidocaine after formalin in the 8th interval showed a significant difference with the control group (**Figure 2**,  $p < 0.01$ ). There was an obvious difference in this group with the control group in this interval, but there was not significant difference between these groups in the 7th interval. However, this difference was significant in the lidocaine-formalin group ( $p < 0.05$ ), possibly due to the number of rats that were studied (**Figure 1**).

**Morphine dependent rats. Saline-saline group.** There was no significant difference between the severity of behavioral response in both the morphine dependent saline groups (with the period of 5 or 9 minutes).

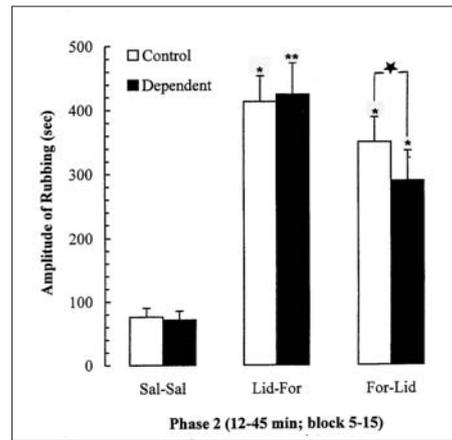
**Saline-formalin and formalin-saline groups or control groups.** The morphine dependent rats that received 50  $\mu$ l of N/S, 5 minutes before and 9 minutes after sc injection of formalin, showed a biphasic nociceptive response with a period of decreased nociceptive reaction between the 2nd and the 5th intervals. However, in these 3 intervals, the behavioral reaction of animals was more than that in the morphine independent groups, but this difference was not significant.

**Lidocaine-formalin group.** In this group, the response to the first phase of noxious stimulus was significantly different than the control group. The first phase was completely abolished. From the 1st to the 4th intervals, there was no significant difference between this group and the saline group. The phase of no reactions was decreased one interval in comparison with the independent group, possibly due to the number of animals (**Figure 1**).

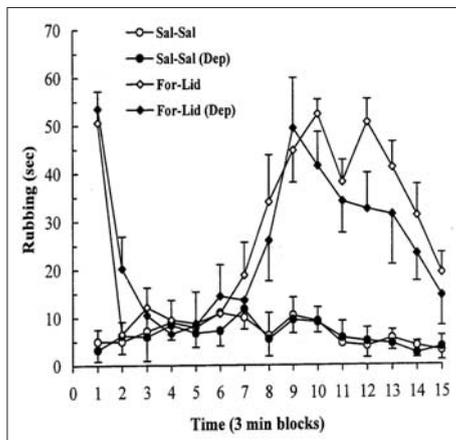
**Formalin-lidocaine group.** In this group, the first phase was the same as the control group, but the second phase was delayed until the 8th interval, and it was significant in comparison to the control



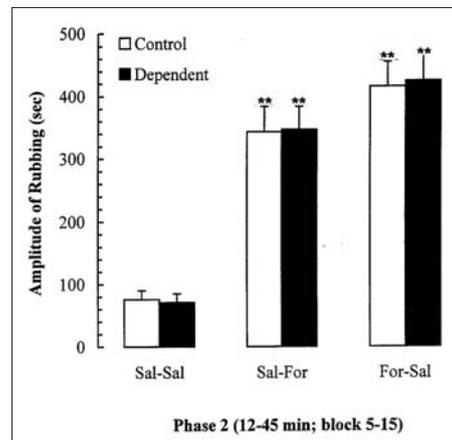
**Figure 1** - Comparison of the rubbing activity caused by the formalin test in the lidocaine-formalin subgroups of both the dependent and independent rates with each other, and the saline group. Sal - saline, Dep - dependent, Lid - lidocaine, For - formalin.



**Figure 3** - Comparing the summation of seconds that the animals spent rubbing the injected area in the dependent and independent lidocaine-formalin and formalin-lidocaine groups with each other, and with the saline group (phase 2). ★ $p<0.05$ : between dependent and independent groups, \* $p<0.05$  and \*\* $p<0.01$  significant differences with saline group.



**Figure 2** - Comparison of the rubbing activity caused by the formalin test in the formalin-lidocaine subgroups of both the dependent and independent rates with each other, and the saline group.



**Figure 4** - Comparing the summation of seconds that the animals spent rubbing the injected area in the dependent and independent control groups with each other, and with the saline group (phase 2). \*\* $p<0.01$  significant differences with saline group.

group ( $p<0.01$ ), and was the same as the saline group. The animals in this group showed less nociceptive response in comparison to the independent group ( $p<0.05$ ) (Figure 2). To show the similarity of the 2 main groups, the summation of the seconds that the animals spent rubbing the injected area have been compared in all similar groups of dependent and independent rats (Figures 3 & 4).

**Discussion.** This study was conducted to ascertain whether the morphine dependent group needs a higher dosage of local anesthetic agents to achieve analgesia. Reviewing the prior research, we were unable to find studies performed directly on this subject, and the available studies only helped us to

choose the method of our study.<sup>10,13</sup> Considering the possibilities we had, this study was performed on N-MRI male rats with formalin test. Each of the 2 main morphine dependent and independent rat groups were divided into subgroups of control (saline-formalin and formalin-saline), receiving lidocaine prior to formalin (lidocaine-formalin group) and receiving lidocaine after formalin (formalin-lidocaine group), in order to be compared with each other, and also with the results of the prior study.<sup>10</sup> The saline groups in both the morphine dependent and independent groups did not show any differences (Figures 1-4). The control groups in both of the 2 main groups showed 2 distinct nociceptive responses that are the same as each other and also the same as the results

of the prior study.<sup>10</sup> The first phase is short and the second phase is long lasting, there is a period with no significant nociceptive response between the 2nd to the 5th period in each group. The severity and the length of each phase are the same as the other group, and no significant differences were found. Reaction to pain between the 2nd and the 5th interval was more severe in the morphine dependent group, but without any statistical difference.

In the morphine dependent and independent lidocaine-formalin groups, the first phase was suppressed compared to the control group ( $p < 0.0001$ ). The period of decreased reaction between the 2 main phases in the dependent group is from the 1st to the 4th intervals, but in the independent group it is from the 1st to the 6th interval, possibly due to the number of rats, and is not observed in other tests, in addition also the form of the figures is the same. In Dallel's study,<sup>10</sup> in the lidocaine-formalin independent group, lidocaine had no effects on the second phase, however, in our study such an effect is obvious, possibly due to the difference between the race and weight of the rats in the 2 studies (Sprague Dawley, 190-220 gr). The lidocaine-formalin group showed a significant difference from the saline group after the 6th interval ( $p < 0.01$ ), however, at the 13th, 14th and 15th intervals, the difference was significant ( $p < 0.05$ ).

The reaction of the animal at the end of the second phase is severer in the lidocaine-formalin group, which is not seen in formalin-lidocaine group. This problem may be due to the number of the rats and their different reactions. In the formalin-lidocaine dependent and independent groups, the first phases are the same as each other, and the same as the control group. The period between the first and the second phases is until the 8th interval, which is significant in the 2 groups in comparison with the control group. However, in the dependent group ( $p < 0.01$ ) and in the independent group ( $p < 0.05$ ), the difference is significant, possibly due to the number of animals. In the comparative study,<sup>10</sup> in the lidocaine-formalin group, only the 5th and the 6th intervals were suppressed, whereas in our study the 7th and 8th intervals are also suppressed. This effect may be due to the difference in animals' race and weight, and is not seen between morphine dependent and independent rats of this study.

The total seconds the animal spends rubbing the injected area in the dependent formalin-lidocaine group in the second phase is less than that of the independent group ( $p < 0.05$ ) (Figure 3), and is not seen in the control groups (Figure 4). Considering the immunosuppression caused by morphine dependency,<sup>14</sup> and the fact that the inflammatory response is also effective in the second phase of

formalin test<sup>15</sup> (besides the central effect), this may be acceptable, however, more investigations are required as this effect is not seen in other groups.

From the results of this study, we can suggest that morphine dependency does not induce the need for higher doses of local anesthetic agents to achieve analgesia. However, this result would be more acceptable if we could do a similar study with a true method in humans.

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