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Differential Effects of Activation of Lumbar and Thoracic 5-$HT_{2A/2C}$ Receptors on Nociception in Rats

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KJØRSVIK, A., R. STØRKSON, A. TJØLSEN AND K. HOLE. *Differential effects of activation of lumbar and thoracic 5-$HT_{2A/2C}$ receptors on nociception in rats.* PHARMACOL BIOCHEM BEHAV **56**(3) 523–527, 1997.—The role of 5-HT_2 receptors in nociceptive behaviour of rats was investigated using spinal administration of the 5-$HT_{2A/2C}$ receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI), the 5-$HT_{2A/2C}$ antagonist ketanserin and the glutamate receptor agonist NMDA. Nociceptive behaviour was scored after injections at upper thoracic or lumbosacral levels. DOI (0.1–10 mM, 15 μl) administered at the upper thoracic level induced pain-like behaviour in a dose-dependent manner and a long-lasting motor depression at the greatest dose. At the lumbosacral level a similar dose-dependent pain-like behaviour was observed, but it was less pronounced. Motor depression was not observed at any dose. Ketanserin injected before DOI blocked both nociceptive and motor effects. Stimulation of both NMDA and 5-$HT_{2A/2C}$ receptors had a mutually potentiating effect. The present results show that the effects of DOI were more pronounced at the upper thoracic than at the lumbosacral level. This is possibly caused by the difference in 5-$HT_{2A/2C}$ receptor density at the two levels. The motor depression induced by the greatest dose of DOI given at the upper thoracic level appears to mask the pain-like behaviour. The nociceptive behaviour seen after DOI injection is further increased following co-injection of NMDA.

Pain Serotonin-2A/2C receptors Spinal cord Behavior Rat

IN THE spinal cord nociceptive signals are regulated through descending pathways. The more important of these systems is the raphe-spinal serotonergic pathway and it has generally been assumed that activation of this pathway inhibits nociception. In recent years it has become clear that there are several subgroups of serotonin receptors (2,5,8) and it is conceivable that these subgroups may not have the same function. Although stimulation of some of these serotonin receptors inhibits nociception, it seems possible that other receptors might increase nociception.

The 5-HT_2 receptor class now comprises three distinct receptor subtypes, namely 5-HT_{2A} (previously 5-HT_2), 5-HT_{2B} (previously 5-HT_{2F}) and 5-HT_{2C} (previously 5-HT_{1C}) (4,5,7,8). The role of 5-HT_2 receptors in nociception has been discussed, and conflicting results have been presented, whether they inhibit (12) or enhance nociceptive transmission (3,14). It has been suggested that different methods probably give conflicting results. Activation of spinal 5- $HT_{2A/2C}$ receptors may produce pro-nociceptive biting and scratching behaviours (12) as well as anti-nociceptive effects (12,15). It has also been shown that the 5-$HT_{2A/2C}$ receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) enhances the nociceptive behaviour elicited by intrathecal administration of the glutamate receptor agonists NMDA (*N*-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid) in mice (6). In a recent study it has been proposed that agonists with high affinity for 5-$HT_{2A/2C}$ receptors, e.g. DOI, even may produce an early pro-nociceptive action followed by secondary antinociceptive effects (14). However, based on the above reports we found it interesting to examine whether differences in receptor density are likely to have functional consequences. The distribution of 5-$HT_{2A/2C}$subtypes in the spinal cord may be an important factor in the effect observed when the receptors are activated. Interestingly, recent studies have shown that different serotonin receptor groups and subgroups show different laminar and rostro-caudal distributions within the spinal cord (4). Thus, a possible explanation of the disagreement concerning the roles of 5-$HT_{2A/2C}$ receptors is that most

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studies have neglected differences in receptor distribution in the spinal cord as a function of cord level. Studies have usually included data from either the cervical, upper thoracic or lumbosacral levels.

Therefore, we chose to study the behavioural response to intrathecal injection of the 5-$HT_{2A/2C}$ agonist (DOI) and the interaction with intrathecal NMDA at two different levels in the spinal cord, the upper thoracic and the lumbosacral. The results of these experiments were compared.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Mol:SPRD, Møllegaard, Denmark), weighing 280–330 g at the time of experiment, were used. The animals were housed at 23 ± 0.5 °C in individual cages (L × W × H: 26 × 20 × 13 cm) with free access to food and water, and were kept on a 12/12 h light/dark cycle with lights on at 0700 AM. Prior to testing, the rats were adapted to the test room for approximately two h. Separate groups of animals were used in different experiments.

Drugs

The 5-$HT_{2A/2C}$ receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine ((±)-DOI HCl; Research Biochemicals International (RBI)), the 5-$HT_{2A/2C}$ antagonist ketanserin tartrate (RBI) and the glutamate agonist *N*-methyl-D-aspartic acid (NMDA, Sigma Chemical Co, USA) were given intrathecally in a volume of 15 μl 0.9% NaCl. The solutions were made the day before experiment, and were frozen until used. The solutions were thawed and allowed to reach room temperature before injection. The doses of DOI and NMDA were based on pilot studies, and were chosen so that the intermediate doses were expected to give a moderate nociceptive response.

Ethical Considerations

The guidelines for investigations in animals, given by The International Association for the Study of Pain (16) were followed, and the experiments were approved by the Norwegian Committee for Experiments on Animals (Utvalg for forsøk med dyr).

Insertion of Catheters with Catheter-Through-Needle Technique, Injection Procedure

The intrathecal injection technique was adapted from a method developed in our laboratory (13). Briefly, the animals were anesthetised with halothane and the catheters were inserted intrathecally between the fifth and sixth lumbar vertebrae. The lumbar puncture was performed using a 20 G cannula through which a polyethylene catheter (PE 10, outer diameter 0.61 mm) was passed. Correct intrathecal localization of the cannula was confirmed by a tail-flick or a paw retraction, flow of cerebrospinal fluid and the easy insertion of a catheter through the cannula.

For upper thoracic injections, the tip of the catheter was inserted as far as to the second thoracic vertebra, which may easily be palpated. For lumbar catheterization the catheter tip was inserted to the level of vertebra L1. The cannula was then withdrawn, leaving the catheter in place. Finally, the catheter was tunnelled subcutaneously, and a knot made on the catheter prevented it from sliding out. The catheter was sealed by melting the end.

The deadspace of the catheter was about 6 μl. The whole procedure of insertion and fixation took about 10 min per animal.

Vascular Perfusion Fixation and Dissection

To confirm the positions of the catheter tips, half the number of the animals used in the thoracic and the lumbar experiments with DOI alone were randomly selected and vascular perfusion fixed with 3% glutaraldehyde in a 0.1 M Sørensen phosphate-buffer (1). After the perfusion, each individual vertebra was identified and the spinal cord segments identified until the rostral end of the catheter was located.

Testing Procedure

Experiments were performed 1–2 days after implantation of catheters. The animals were placed individually in standard macrolone cages (L × W × H: 26 × 20 × 13 cm) after the implantation to prevent them damaging the catheters of other animals. Two minutes before drug injection the animal was placed in a transparent observation chamber (L × W × H: 36 × 24 × 30 cm) with wooden chips on the floor. Ambient temperature during testing was 21–23 °C.

Drugs were injected intrathecally through the catheter in a volume of 15 μl for each drug in all experiments. After the i.th. injection, the animal was returned to the observation chamber and the amount of time the animal spent biting and scratching the front limbs or hind limbs and the degree and time of motor depression was scored during the initial fifteen min after drug injection. The observer was unaware of the drug treatment of the animal.

Scored Behavioral Categories

Category 1. Grooming of the snout.

Category 2. Licking or biting the front or hind limbs, scratching paw against the body or intense grooming of the body or the head.

Category 3. Flat-body posture or hindlimb abduction (motor depression).

DOI Dose-Response Study

Four groups of six animals were used. The groups received saline, 0.1, 1.0 or 10 mM DOI. Four animals were tested on each of six consecutive days, and each day one animal from each group was tested.

DOI Combined With Ketanserin

Four groups of five animals were used. The groups received saline + saline, saline + 1.0 mM DOI, 1.0 mM ketanserin + saline or 1.0 mM ketanserin + 1.0 mM DOI. The two injections were given intrathecally through the catheter with an interval of 1 min. The experimental procedure was similar to that of the DOI experiment described above.

DOI Injected With NMDA

Four groups of five animals were used. The groups recived saline + saline, saline + 0.1 mM DOI, saline + 0.1 mM NMDA or 0.1 mM DOI + 0.1 μM NMDA. The two drugs given in each group were injected intrathecally through the catheter with an interval of 2 min.

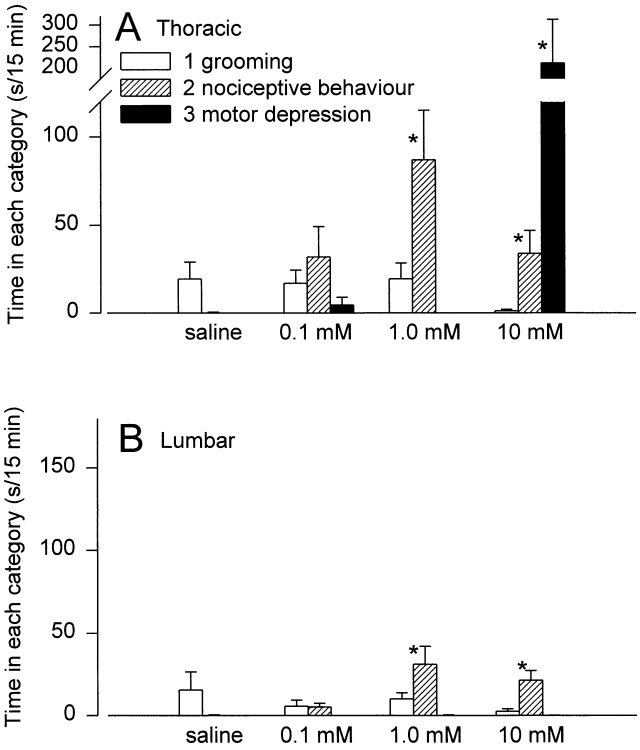

FIG. 1. The behavioural response to intrathecal injection of saline and different doses of DOI at the level of the upper thoracic (A) and lumbosacral (B) levels of the cord. Means ± SEM; $n = 6$ in each group. *$p < 0.05$, **$p < 0.01$, Mann–Whitney test vs. saline group.

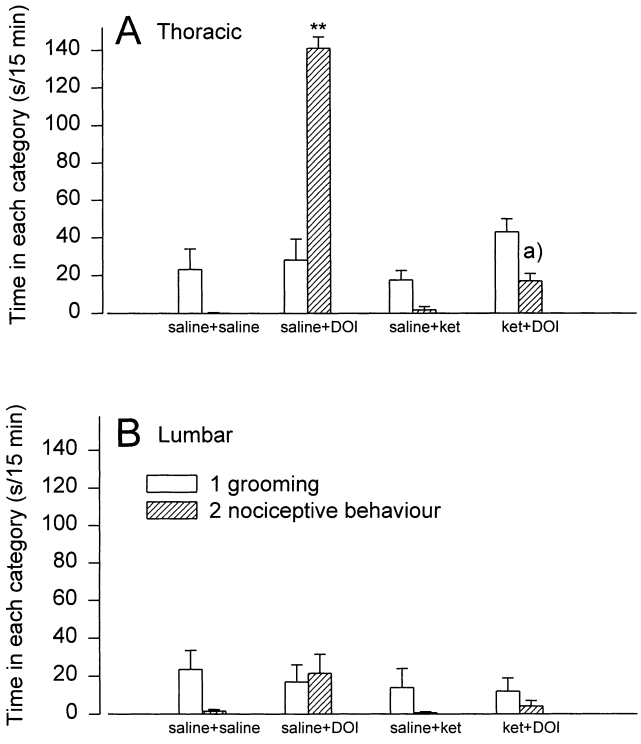

FIG. 2. The response to intrathecal injection of DOI (1.0 mM), ketanserin (ket, 1.0 mM) or the combination of the two drugs at both the upper thoracic (A) and lumbosacral (B) levels. Means ± SEM; $n = 5$ in each group. *$p < 0.05$, **$p < 0.01$, Mann–Whitney test vs. saline group. a) $p < 0.05$, Mann–Whitney test vs. DOI group.

STATISTICAL ANALYSIS

The dose-response relationship was evaluated using one-way analysis of variance (ANOVA). For evaluation of the co-administration effects, two-way ANOVA was applied. Mann–Whitney rank-sum tests were used when the comparison was restricted to two means.

RESULTS

DOI Given Alone

The behaviour elicited by injection of DOI at the upper thoracic segment of the spinal cord is shown in Fig. 1A. Doses in the range 0.1–10 mM DOI (15 μl) induced dose-dependent biting and scratching of the fore limbs and digits, interpreted as a nociceptive response (category 2: $F(3,20) = 4.10$, $p = 0.020$, ANOVA). At 10 mM DOI nociceptive behaviour, hind limb abduction and flat body posture was observed.

The behaviour scored after injection of DOI at the lower lumbar segments of the spinal cord is shown in Fig. 1B. DOI induced a pain-like behaviour with biting and scratching of the back and the hind limbs after injection of doses in the range 0.1–10 mM DOI in 15 μl (category 2: $F(3,16) = 5.12$, $p = 0.011$, ANOVA). The nociceptive response was not as strong as observed in the thoracic experiment. Hind limb abduction or flat body posture was not observed.

Dissection showed that catheters aimed at the second thoracic vertebra were placed adjacent to vertebrae T2 or T3. In ten of the twelve animals dissected the catheters were located at the level of the second vertebrae and in two of the animals the catheters were located at the upper part of the third thoracic vertebra. In this part of the spinal cord, the segments correspond very well to the vertabrae, hence the catheter tips were located at the level of spinal segment T2 or T3. Dissection of animals with lumbar catheters showed that in eight of the animals the catheters were located at the level of the first lumbar vertebra and in four animals the catheters were located at the upper part of the second lumbar vertebra. These two vertebrae correspond to spinal segments L3, L4, L5 and S1 (R. Bjugn, personal communication).

DOI Injected With Ketanserin

The behaviour after the administration of both ketanserin and DOI at the upper thoracic and the lower lumbar levels of the spinal cord is shown in Fig. 2A and Fig. 2B. Ketanserin showed a tendency to block the nociceptive behaviour induced by 1.0 mM DOI, which was clearly significant at the thoracic level (2-way ANOVA: Category 2, thoracic level: DOI effect: $F(1,16) = 18.90$, $p < 0.001$, ketanserin effect: $F(1,16) = 11.57$, $p = 0.004$, DOI × ketanserin interaction: $F(1,16) = 12.13$, $p = 0.003$. Lumbar level: DOI effect: $F(1,16) = 4.62$, $p = 0.047$, ketanserin effect: $F(1,16) = 2.81$, $p = 0.11$, DOI × ketanserin interaction: $F(1,16) = 2.28$, $p = 0.15$). Alone, 1.0 mM ketanserin gave no pain-like behaviour or motor depression. This was observed at both the thoracic and lumbar levels of the spinal cord.

DOI Injected With the Glutamate-Agonist NMDA

The behaviour after the administration of both DOI and NMDA at the upper thoracic and lower lumbar levels of the

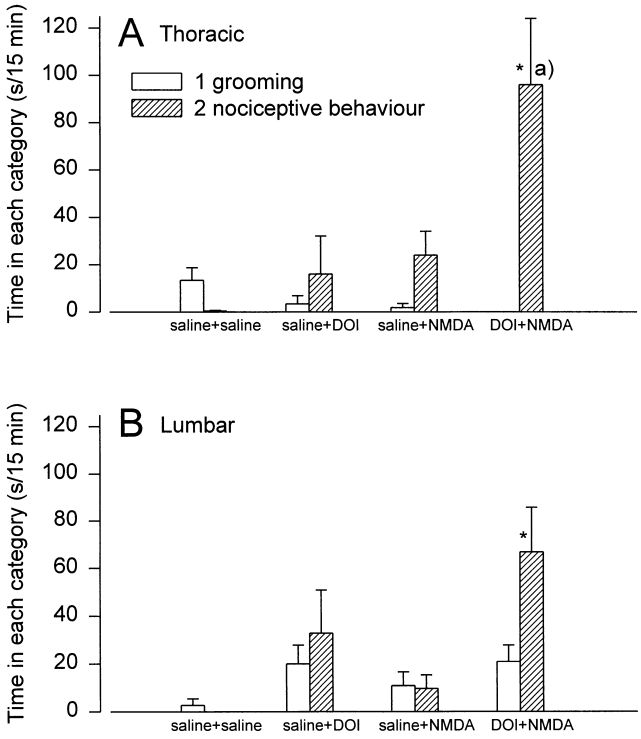

FIG. 3. The response to intrathecal injection of DOI (0.1 mM), NMDA (100 nM) or the combination of the two drugs at the upper thoracic (A) and lumbosacral (B) levels. Means ± SEM; $n = 4$ in each group. *$p < 0.05$, **$p < 0.01$, Mann–Whitney test vs. saline group, a) $p < 0.05$, Mann–Whitney test vs. DOI group.

spinal cord is shown in Fig. 3A and Fig. 3B. Given separately, 0.1 μM NMDA and 0.1 mM DOI gave little or no nociceptive behaviour. The administration of both NMDA and DOI induced an intense pain-like behaviour in both experiments greater than when the same doses of DOI or NMDA were given separately, although the interaction did not reach statistical significance (2-way ANOVA: Category 2, thoracic level: DOI effect: $F(1,12) = 6.73$, $p = 0.024$, NMDA effect: $F(1, 12) = 9.44$, $p = 0.010$, DOI × NMDA interaction: $F(1,12) = 2.75$, $p = 0.12$. Lumbar level: DOI effect: $F(1,12) = 11.61$, $p = 0.005$, NMDA effect:$F(1,12) = 2.76$, $p = 0.12$, DOI × NMDA interaction: $F(1,12) = 0.86$, $p = 0.37$).

DISCUSSION

The most important finding in this study is that intrathecal injection of a 5-$HT_{2A/2C}$ receptor agonist induced nociceptive behaviour in a dose-dependent manner. This nociceptive behaviour was directed specifically towards the dermatomes corresponding to the segments of the spinal cord where the tips of the injection catheters were located. The behaviour was also effectively blocked by intrathecal injection of the 5-$HT_{2A/2C}$ receptor antagonist ketanserin.

We found that the response was more pronounced at the upper thoracic segments than at the lumbosacral segments. This finding may be due to the different density of the 5-$HT_{2A/2C}$ receptors at these two levels, or to the ratio between 5-HT_{2A} and 5-HT_{2C} receptor subgroups. Both receptor binding and autoradiographic experiments have shown that the 5-$HT_{2A/2C}$ receptors are present in the dorsal horn (4). In addition, 5-HT_{2A} receptors are present predominantly at the upper thoracic level, mainly in lamina X. The 5-HT_{2C} receptor subgroup is on the other hand concentrated in laminae V and VII, and more evenly distributed in the rostro-caudal direction.

Since they most certainly differ in spinal distribution, they may give rise to different response to stimulation of both 5-HT_{2A} and 5-HT_{2C} receptors at different spinal levels. This is also in agreement with the increased response that was observed in the present study when DOI was injected at the upper thoracic level where the density ratio of 5-HT_{2A} relative to 5-HT_{2C} receptors is high. However, several other possibilities might be involved in the response, and further investigations have to be done.

Other workers have found that stimulation of 5-$HT_{2A/2C}$-receptors may induce antinociception (12,15). The question of the dual effects of 5-$HT_{2A/2C}$ receptor stimulation on nociceptive modulation can be approached with the possibility that 5-HT_{2A} and 5-HT_{2C} receptors mediate different effects.

Previous studies have indicated that activation of spinal 5-HT_{2A} receptors enhances transmission of nociceptive impulses, possibly due to an increased release of substance P from presynaptic terminals in the spinal cord (3). On the other hand, spinal antinociception may be mediated by activation of the 5-HT_{2C} receptors that modulate noradrenaline release from the terminals in the spinal cord (9,10). However, 5-HT_{2C} activation has also been shown to have dual effects on noradrenaline release, inhibiting the release at lower concentrations but enhancing it at higher concentrations (9). The parallel distribution of receptors between substantia gelatinosa and lamina X further suggests that serotonin and noradrenaline may have parallel functions in these regions of the spinal cord (11). Thus, it seems reasonable that the ratio between 5-HT_{2A} and 5-HT_{2C} is important to take in consideration since these two receptor subgroups may mask the response of each other.

Our experiments showed that activation of spinal 5-$HT_{2A/2C}$ receptors increased the nociceptive response in a dose-dependent manner up to a certain dose level at both levels studied. This behavioural response was restricted to the dermatomes corresponding to the level of injection. However, at higher doses (⩾10 mM, 15 μl), DOI induced obvious motor effects, and the nociceptive response was decreased in both intensity and duration. It seems that at higher doses of DOI the motor depression masks the pain-like behaviour. Although the motor effects were confined to the hind parts of the body, they were seen only after thoracic injections, suggesting an indirect action on lumbar motor circuits, possibly after redistribution of DOI to supraspinal motor areas.

In this study, it was observed that doses of DOI reported to be antinociceptive at the lumbar level (12) induce nociceptive behaviour when administered thoracically. This supports the notion that the 5-HT_{2A} receptor distribution is important in determining the response to intrathecal administration of DOI.

The nociceptive behaviour elicited by DOI was effectively reduced by intrathecal administration of the 55-$HT_{2A/2C}$ antagonist ketanserin, strongly indicating that this segmentally directed behaviour was specifically caused by stimulation of 5-$HT_{2A/2C}$ receptors.

When DOI was co-administered with NMDA, an increase in the biting and scratching behaviour was observed. This shows that co-stimulation of NMDA and 5-$HT_{2A/2C}$ receptors seems to have at least an additive effect, further suggesting that 5-$HT_{2A/2C}$ receptors are involved in enhancement of nociception. No motor depression was observed when DOI was

administrated with NMDA in these doses, indicating that the motor effect of DOI is separate from the effect on nociception.

To conclude, the effect observed after activation of the 5-$HT_{2A/2C}$ receptors with the agonist DOI was more pronounced at the upper thoracic segments than at the lumbosacral segments. These results may be explained by taking into consideration the distribution of the 5-$HT_{2A/2C}$ receptors in the spinal cord. The concentration-ratio between 5-HT_{2A} and 5-HT_{2C} is more marked at the upper thoracic level than at lumbosacral level. Thus, the enhanced nociceptive response observed at upper thoracic levels may be a consequence of the increased effect of 5-HT_{2A} receptor activation due to a high 5-HT_{2A} receptor concentration. It also seems probable that the reduction of pain-like behaviour after a high dose of DOI is caused by a masking of the nociceptive response by motor depression produced by a supraspinal redistribution of DOI.

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