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# Effects of the Putative 5-$HT_{1A}$ Receptor Agonist 8-OH-2-(di-n-propylamino)Tetralin on Nociceptive Sensitivity in Mice

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FASMER, O. B., O.-G. BERGE, C. POST AND K. HOLE. *Effects of the putative 5-$HT_{1A}$ receptor agonist 8-OH-2-(di-n-propylamino)tetralin on nociceptive sensitivity in mice.* PHARMACOL BIOCHEM BEHAV **25**(4) 883–888, 1986.—The ability of 8-OH-2-(di-n-propylamino)tetralin (8-OH-DPAT) to alter nociceptive sensitivity in mice was studied using the tail-flick, hot-plate and formalin tests. Subcutaneous (SC) administration of 8-OH-DPAT (0.63–1.0 mg/kg) dose-dependently increased the temperature at which hindpaw lick occurred in a hot-plate test using slowly rising temperature and increased the latencies to hindpaw lick, but reduced the latencies to jump in a conventional hot-plate test. Intracerebroventricular (ICV) injections (0.25–1.0 μg) produced similar results in the conventional hot plate test. Following intrathecal (ITH) injections (0.25–1.0 μg), however, the latencies to hindpaw lick were elevated without any change in jump latencies. In the formalin test a low systemic dose of 8-OH-DPAT (0.063 mg/kg) elicited hyperalgesia, while hypoalgesia was found after a high dose (1.0 mg/kg). ICV injection of 1.0 μg produced hypoalgesia in the formalin test while the same dose injected ITH was without effect. 8-OH-DPAT did not alter tail-flick latencies, either by SC, ICV or ITH administration. Previous studies have shown that 8-OH-DPAT stimulates central serotonergic receptors, and shows selectivity for the 5-$HT_{1A}$ recognition site. The present findings indicate an involvement of 5-$HT_{1A}$ receptors in the processing of nociceptive information both at spinal and supraspinal sites. However, stimulation of 5-$HT_{1A}$ receptors does not seem to affect spinal, nociceptive reflexes.

8-OH-DPAT 5-Hydroxytryptamine Nociception Mice

BOTH ascending and descending serotonergic pathways have been implicated in the central regulation of nociceptive sensitivity. Systemic administration of serotonergic agonists or injection of serotonin into either the cerebral ventricles or the spinal subarachnoid space reduce behavioural and electrophysiological responses to noxious stimulation [2, 3, 19, 20, 25, 30]. Lesioning of descending serotonergic systems or intrathecal (ITH) administration of serotonergic antagonists elicit hyperalgesia [4, 13, 26].

Serotonergic receptors in the central nervous system have been classified into two types, 5-$HT_1$ and 5-$HT_2$, on basis of receptor binding affinity of labeled serotonin and spiperone [24]. Recently it has been shown that it is possible to discriminate further between two subtypes of the 5-$HT_1$ recognition site, 5-$HT_{1A}$ and 5-$HT_{1B}$ [21], and there may even be one more subtype [11]. Tritiated 8-OH-2-(di-n-propylamino)-tetralin (8-OH-DPAT) has been reported to bind with high selectively to the 5-$HT_{1A}$ site [22]. In biochemical and behavioural studies 8-OH-DPAT elicits changes indicating central serotonergic receptor stimulation [16].

The purpose of the present study was to examine changes in nociceptive sensitivity following systemic and central administration of 8-OH-DPAT. The formalin, tail-flick, and hot-plate tests were used in order to evaluate the effect of 8-OH-DPAT both on a spinal nociceptive reflex and on more complex behavioural responses to noxious stimulation.

## METHOD

### *Animals*

Male albino NMRI mice (30–40 g, Møllegård, Denmark) were used. The animals were housed in colony cages and had free access to food and water. Testing took place during the light phase of a 12/12 hr light/dark cycle.

### *Injection Procedures*

8-OH-DPAT HBr was dissolved in 0.9% NaCl and administered subcutaneously (SC) in the neck (0.063–1.0 mg/kg), intracerebroventricularly (ICV, 0.25–1.0 μg) or

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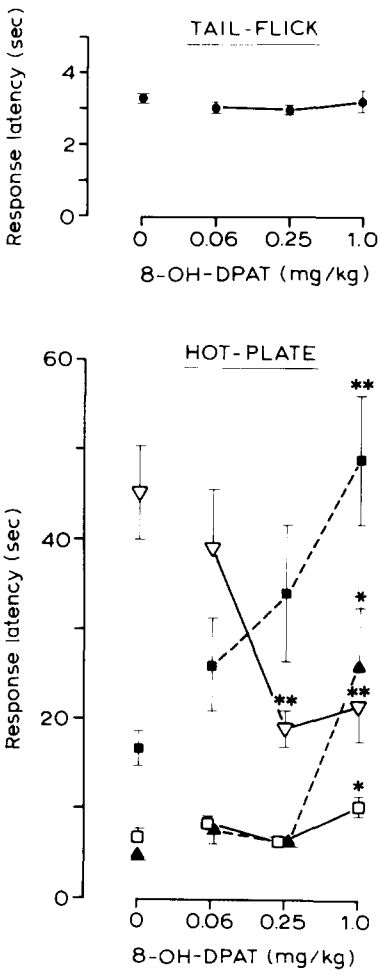


FIG. 1. Effects of 8-OH-DPAT in the tail-flick and conventional hot-plate tests 30 min after subcutaneous administration (n=8). Four response latencies were recorded in the hot-plate test: Shaking or kicking of one hindlimb (□), forepaw lick (▲), hindpaw lick (■) and jump (▽). The doses are plotted on a log scale. Results are presented as mean±SEM. $*p<0.05$, $**p<0.01$, compared to saline treated group, Dunnett's test.

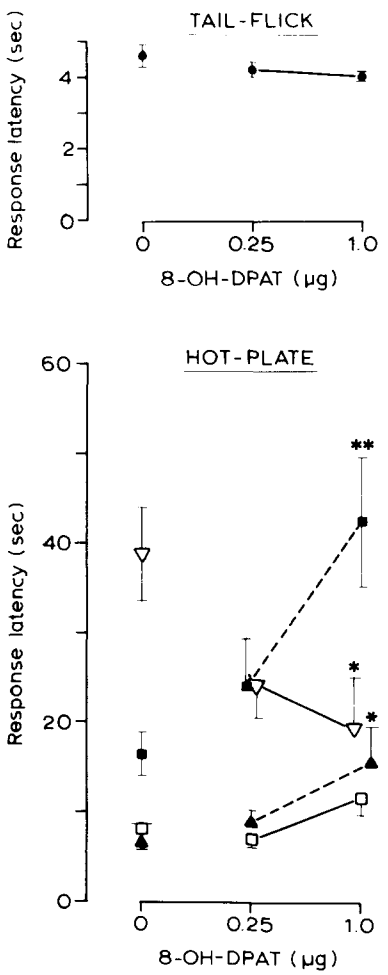


FIG. 2. Effects of 8-OH-DPAT in the tail-flick and conventional hot-plate tests 10 min after intracerebroventricular administration (n=7–8). Four response latencies were recorded in the hot-plate test: Shaking or kicking of one hindlimb (□), forepaw lick (▲), hindpaw lick (■) and jump (▽). The doses are plotted on a log scale. Results are presented as mean±SEM. $*p<0.05$, $**p<0.01$, compared to saline treated groups, Dunnett's test.

intrathecally (ITH, 0.25–1.0 μg). The control groups received an equal volume of vehicle (0.9% NaCl). Subcutaneous injection volume was 10 ml/kg.

ICV injections were performed using a modification of the procedure described by Haley and McCormick [15]. The injection site was 2 mm from the midline on a line drawn through the anterior base of the ears. A gauge 26 cannula was inserted to a depth of 3.5 mm from the surface of the skull and a volume of 5 μl was injected.

The ITH injection technique was adapted from the method described by Hylden and Wilcox [18]. An incision was made in the skin and a lumbar puncture performed using a gauge 30 needle connected to a microsyringe with polyethylene tubing. The needle was inserted between L5 and L6, and a volume of 5 μl was injected as a bolus injection (injection time less than 5 sec).

*Nociceptive Testing*

The tail-flick, hot-plate and formalin tests were employed in order to measure nociceptive sensitivity in mice after administration of 8-OH-DPAT. One hour before testing, the animals were placed individually in standard macrolone cages (30×12×13 cm), which also served as observation chambers in the formalin test. SC injections were performed 30 min, and ICV and ITH injections 10 min before testing. The same groups of mice were used in the tail-flick and hot-plate tests. Hot-plate testing was performed immediately after the tail-flick latencies had been recorded. Separate groups were used in the formalin test. Testing was conducted by an observer ignorant as to the drug treatment of the animals.

Testing of tail-flick latencies was performed using an HTC Inc. Mod. 33 Analgesiameter, with the light beam focused on the tip of the tail [9].

For conventional hot-plate testing a modification of the method of Woolfe and MacDonald [29] was used. An HTC Inc. Mod. 35-D Analgesiameter was set to a temperature of 55±0.2°C. Four response latencies were recorded for each animal: shaking or kicking of one hindlimb (shake), forepaw

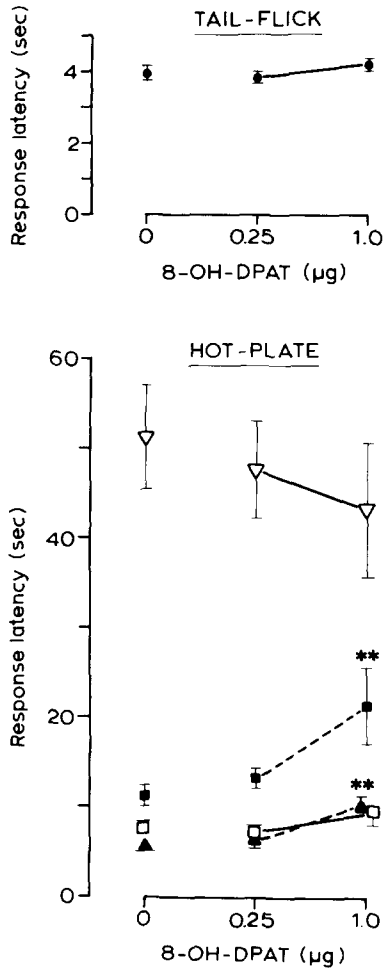


FIG. 3. Effects of 8-OH-DPAT in the tail-flick and conventional hot-plate tests 10 min after intrathecal administration (n=7–8). Four response latencies were recorded in the hot-plate test: Shaking or kicking of one hindlimb (□), forepaw lick (▲), hindpaw lick (■) and jump (▽). The doses are plotted on a log scale. Results are presented as mean±SEM. $^{**}p<0.01$, compared to saline treated group, Dunnett's test.

lick, hindpaw lick and jump. A cut-off time of 60 sec was employed. The rising temperature hot plate test [23] was conducted with the same apparatus. The temperature was increased from 43°C at a rate of approximately 2.5°C/min. The temperature at which a hindpaw lick occurred was recorded.

The formalin test was adapted from the method described by Dubuisson and Dennis [10]. Twenty μl of 1% formalin was injected into the dorsal surface of the right hindpaw. The mice were observed for 2 min after the injection of formalin, and the amount of time spent licking the injected hindpaw was measured.

*Statistics*

Unless otherwise stated, $p$-values refer to Dunnett's test subsequent to one-way analysis of variance (ANOVA). Statistical significance was accepted at the 5% level.

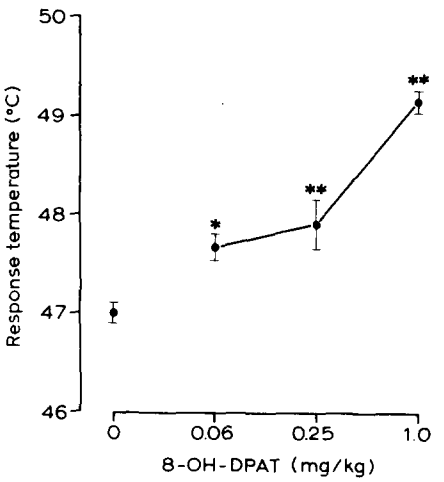


FIG. 4. Effects of 8-OH-DPAT in the rising temperature hot-plate test 30 min after subcutaneous administration. The doses are plotted on a log scale. Results are presented as mean±SEM (n=8). $^{*}p<0.05$, $^{**}p<0.01$, compared to saline treated group, Dunnett's test.

## RESULTS

There were no overt behavioural effects of systemic or central administration of 8-OH-DPAT. In particular, no apparent signs of a 5-HT syndrome could be observed.

*Tail-Flick Test*

There were no significant changes in tail-flick latencies after administration of 8-OH-DPAT SC (0.063–1.0 mg/kg, Fig. 1), ICV (0.25–1.0 μg, Fig. 2) or ITH (0.25–1.0 μg, Fig. 3).

*Conventional Hot-Plate Test*

Subcutaneous injection (Fig. 1) of 1.0 mg/kg of 8-OH-DPAT increased the latencies to shake (48% increase compared to controls, $p<0.05$). Following ICV (Fig. 2) or ITH (Fig. 3) administration of 1.0 μg the latencies to shake were also somewhat increased (43 and 25%), but these changes were not statistically significant.

Subcutaneous injection of 1.0 mg/kg elicited a large increase in the latencies to forepaw lick (433%, $p<0.01$, Fig. 1). Increased forepaw lick latencies were also found after ICV (149%, $p<0.05$, Fig. 2) and ITH (77% $p<0.01$, Fig. 3) administration of 1.0 μg.

Latencies to hindpaw lick were increased dose-dependently after SC administration of 0.063–1.0 mg/kg, 53–191%, $F(3,27)=5.13$, $p<0.01$, ANOVA. Application of Dunnett's test showed, however, that only the 1.0 mg/kg dose produced results significantly different from vehicle ($p<0.01$). After ICV injections (0.25–1.0 μg), the hindpaw lick latencies were similarly increased, 48–163%, $F(2,23)=6.31$, $p<0.01$, ANOVA, but only the 1.0 μg dose was significantly different from vehicle ($p<0.01$). Intrathecal injection of 1.0 μg increased the hindpaw lick latencies by 88% ($p<0.01$).

Jump latencies were significantly reduced after SC administration of 0.25 and 1.0 mg/kg (58 and 52%, $p<0.01$). Intracerebroventricular administration of 0.25 and 1.0 μg also re-

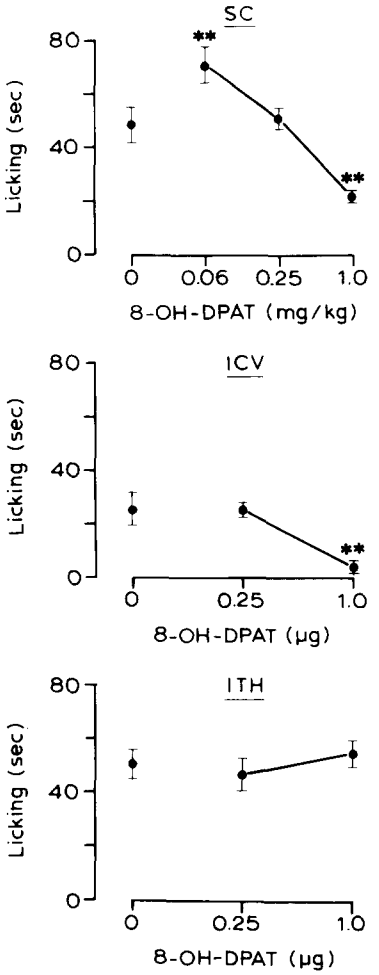


FIG. 5. Effects of 8-OH-DPAT in the formalin test 30 min after subcutaneous (SC) administration and 10 min after intracerebroventricular (ICV) and intrathecal (ITH) administration (n=7–8). The amount of time spent licking the formalin-injected paw was recorded for 2 min after injection of formalin. The doses are plotted on a log scale. Results are presented as mean±SEM. $^{**}p<0.01$, compared to saline treated group, Dunnett's test.

duced the jump latencies, 38 and 49%, $F(2,23)=3.91$, $p<0.05$, ANOVA, but only the 1.0 μg dose was significantly different from vehicle ($p<0.05$). After ITH injections of 0.25–1.0 μg the latencies to jump were not significantly altered.

*Rising Temperature Hot-Plate Test*

The rising temperature hot-plate test was primarily conducted because of the divergent effects of 8-OH-DPAT on the hindpaw lick and jump responses in the conventional hot-plate test. Only SC injections were performed. Jumping prior to hindpaw lick was observed in 4 animals after the lowest dose of 8-OH-DPAT, and in 1 animal in each of the remaining groups (total number of animals was 8 in each group). The hindpaw lick temperatures of these animals were within the range of scores of the mice that did not jump. ANOVA demonstrated significant difference between the groups, $F(3,28)=23.54$, $p<0.001$, each of the 8-OH-DPAT treated groups having significantly higher response temperatures than the controls (Fig. 4).

*Formalin Test*

Subcutaneous administration of 8-OH-DPAT (0.063–1.0 mg/kg) elicited significant changes in the formalin test, $F(3,28)=18.67$, $p<0.01$, ANOVA, Fig. 5. Injection of 0.063 mg/kg increased the amount of licking (46% increase compared to controls, $p<0.01$, Dunnett), 0.25 mg/kg had no effect and 1.0 mg/kg reduced the amount of licking (56%, $p<0.01$, Dunnett). After ICV injection of vehicle the licking response was reduced compared to SC injection of vehicle (25±6 sec and 48±6 sec respectively, mean±SEM), indicating that the ICV injection procedure in itself has an antinociceptive effect in the formalin test. However, administration of 1.0 μg ICV still had a strong antinociceptive effect compared to controls (84% reduction of the licking time, $p<0.01$, Dunnett).

The licking responses were not altered after ITH administration of 0.25–1.0 μg.

## DISCUSSION

The present study demonstrates that 8-OH-DPAT alters behavioural responses to noxious stimulation in the hot-plate and formalin tests. It has previously been shown that 8-OH-DPAT is a potent and selective central 5-HT receptor agonist [16]. Taken together with the reported affinity of 8-OH-DPAT for the $5\text{-HT}_{1A}$ recognition site [22] the present findings suggest that $5\text{-HT}_{1A}$ receptors are involved in the central processing of nociceptive information.

In the conventional hot-plate test the change in hindpaw lick latency indicates that 8-OH-DPAT reduces pain sensitivity, since the latency was increased after SC, ICV and ITH injections. This conclusion is supported by the similar effect on the forepaw lick latency, although this response may be a less reliable index of antinociceptive effect [5]. Also the latency to shake tended to be increased after administration of 8-OH-DPAT, although significantly so only after injection of the highest dose. On the other hand, the latencies to jump were reduced after SC and ICV administration. Thus, if only one response criterion had been employed, the conclusion reached would depend upon whether a lick response or jump had been chosen. This emphasizes the importance of using more than one response criterion in the conventional hot-plate test [13].

In the formalin test, biphasic effects of 8-OH-DPAT were seen. A low systemic dose (0.063 mg/kg), which elicited a small elevation of hindpaw lick latencies and response temperature in the hot-plate tests, increased the amount of licking in the formalin test, while a high dose (1.0 mg/kg), which had a strong hypoalgesic effect as measured with the hindpaw lick in the hot-plate tests, produced a similar hypoalgesia in the formalin test. The hypoalgesia produced by 8-OH-DPAT in the formalin test is apparently mediated by supraspinal 5-HT receptors, since hypoalgesia was elicited by ICV, but not ITH administration.

After ITH injection of 8-OH-DPAT there was a significant increase in the latencies to hindpaw and forepaw lick in the conventional hot-plate test, without any change in the latencies to jump. These findings indicate that 8-OH-DPAT, by an action in the spinal cord, is able to reduce behavioural responses to a noxious thermal stimulus. The lack of effect in

the formalin test after ITH injections may either indicate that in the spinal cord 8-OH-DPAT only affects responses to thermal and not to chemical nociceptive stimuli, or that only the response threshold is altered, while the response to continuous, supra-threshold pain is not measurably affected.

The effects of 8-OH-DPAT in the conventional hot-plate test following SC and ICV administration are open to alternative interpretations. 8-OH-DPAT reduced the latency to jump, but increased the latencies to both forepaw and hindpaw lick. 8-OH-DPAT has been reported to elicit a 5-HT-like behavioural syndrome in rats [16]. Although no overt 5-HT syndrome was observed in mice with the doses used, it is still conceivable that motor effects of 8-OH-DPAT may have interfered with the expression of the behavioural responses. After ICV injection of 1.0 $\mu$g, 5 out of 8 mice failed to show hindpaw lick within the cut-off time of 60 sec, but all of the animals licked the forepaws, suggesting that motor performance was not seriously impaired. Svensson and Alhenius have reported that 8-OH-DPAT increases the acoustic startle response of rats when given in doses from 0.25–2.0 mg/kg [27]. Behavioural excitation, including hyperlocomotion, has also been reported [28]. It is therefore possible that the shortened latencies to jump are related to an enhancement of behavioural reactivity rather than to an increase in nociceptive sensitivity. Although it is possible that the increase in jumping behaviour may have interfered with paw-licking in the conventional hot plate test, this was clearly not the case in the rising temperature paradigm. Furthermore, in the formalin test, paw-licking was also reduced after ICV administration of 8-OH-DPAT. Taken together the present findings suggest that the supraspinal effect of 8-OH-DPAT is to reduce both responses to thermal pain of threshold intensity and responses to chemical pain of longer duration.

Confirming a previous study [7], no effect of 8-OH-DPAT on tail-flick latencies was found. It is well documented that stimulation of serotonergic receptors in the spinal cord may elevate tail-flick latencies [3, 6, 17, 30]. It is therefore possible that the spinally mediated effect of 5-HT agonists on the tail-flick response reflects an activation of receptors not belonging to the 5-$HT_{1A}$ population. Alternatively, higher doses of agonists may be required than to alter hot-plate response latencies [19].

Receptor binding studies with tritiated 8-OH-DPAT have revealed that 8-OH-DPAT binds to both pre- and postsynaptic 5-$HT_1$ receptors [14,22]. Behavioural evidence indicates that stimulation of postsynaptic 5-HT receptors elicits hypoalgesia [3]. It is therefore possible that stimulation of postsynaptic 5-$HT_{1A}$ receptors may explain the observed antinociceptive effects of 8-OH-DPAT.

Reduced serotonergic neurontransmission found after stimulation of 5-HT autoreceptors may be associated with increased nociceptive sensitivity [8]. In the formalin test the hyperalgesia after a low systemic dose of 8-OH-DPAT may possibly reflect reduced serotonergic neurotransmission caused by preferential stimulation of presynaptic 5-HT autoreceptors. Supportive evidence for this hypothesis is the finding that tritiated 8-OH-DPAT binds to presynaptic autoreceptors in the striatum [14]. Furthermore, administration of 8-OH-DPAT in the male rat stimulates sexual behaviour, which may be due to a presynaptic effect, since sexual behaviour is normally supressed by serotonergic systems [1].

On the other hand, it has been reported that reduced serotonergic neurotransmission in the brain may be associated with hypoalgesia [31]. Furthermore, the 5-HT receptor antagonist metergoline elicits hypoalgesia in the hot-plate test when administered ICV [12]. Therefore it cannot be excluded that even the reduced nociceptive sensitivity found in the present study may be related to an effect of 8-OH-DPAT on presynaptic 5-HT autoreceptors.

Thus, a complex role of the 5-HT systems in regulation of nociception is now emerging. Although the more frequent effect of an increase in activity in the 5-HT systems is a reduced pain sensitivity, it seems possible that subdivisions of these systems may have an opposite effect. Also, the consequence of 5-HT stimulation may be different in different behavioural situations and for different stimuli.

The present data suggest that stimulation of 5$HT_{1A}$ receptors affects behavioural responses to noxious stimulation in the mouse hot-plate and formalin tests, while the tail-flick reflex seems to be unaffected. The findings indicate an involvement of 5-$HT_{1A}$ receptors in the processing of nociceptive information both at spinal and supraspinal sites.

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