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Short communication

Antinociceptive effect of a κ-opioid receptor agonist that minimally crosses the blood-brain barrier (ICI 204448) in a rat model of mononeuropathy

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Abstract

The antinociceptive effect of intraplantar (i.pl.) ICI 204448 ((*R,S*)-*N*-[2-(*N*-methyl-3,4-dichloro-phenylacetamido)-2-(3-carboxyphenyl)-ethyl]pyrrolidine hydrochloride)) (20, 30, 40 and 50 μg), a κ-opioid receptor agonist which has limited access to the central nervous system, was studied in a well-established rat model of peripheral mononeuropathy produced by moderate constriction of the sciatic nerve. Vocalization thresholds to paw pressure were used as a nociceptive test. On the injected nerve-injured paw, ICI 204448 at 20 and 30 μg had no significant effect, but higher doses (40 μg) produced a significant antinociceptive effect, which plateaued at 50 μg. By contrast, no antinociceptive effect was observed on the contralateral paw. The effect of ICI 204448 (40 μg) was significantly antagonised by the specific κ-opioid receptor antagonist nor-binaltorphimine (20 and 30 μg), when co-injected in the nerve-injured paw.

Keywords: Mononeuropathic rat; Paw pressure; κ-Opioid receptor agonist (ICI 204448); Peripheral antinociception

1. Introduction

The antinociceptive effects of exogenous as well as endogenous opioids are classically associated with activation of opioid receptors located in the central nervous system. Over the last 10 years, however, attention has also focused on the role of the opioid receptors located on peripheral terminals of primary afferent neurones (Fields et al.,1980; Young et al., 1980). There is increasing evidence supporting a peripheral antinociceptive site of action of opioids in hyperalgesic inflammatory conditions in animals (Refs. in Stein et al., 1989; Kayser et al., 1995). In a rat model of peripheral mononeuropathy, produced by four loose ligatures around the common sciatic nerve and shown to produce abnormalities in pain sensation reminiscent of those seen in man (Bennett and Xie, 1988; Attal et al., 1990), it has been clearly established that morphine and selective opioid receptor agonists administered systemically induce potent and dose-dependent antinociceptive effects on the abnormal reactivity to a mechanical stimulus (Attal et al., 1991; Desmeules et al., 1993), which seems to contradict the classical view that neuropathic pain is opioid resistant (Refs. in Attal et al., 1991). Irrespective of the opioid used, the antinociceptive action was more marked on the paw on the nerve-injured side than on the paw of the contralateral side. In sharp contrast, morphine and selective opioid receptor agonists were ineffective on the abnormal reactivity to thermal stimulation below or at the level of the activation threshold in normal conditions (Lee et al., 1994). It should be noted that these effects were observed 2 weeks after the sciatic injury, when the behavioural pain-related disorders have reached a maximum (Attal et al., 1990). One contributory mechanism could be a peripheral action of the opioid receptor agonists on the nerve-injured paw. This hypothesis was confirmed (Kayser, 1994) using systemic morphine and extremely low doses of local naloxone or its quaternary salt (naloxone methiodide), which has a low capacity to cross the blood-brain barrier (Refs. in Kayser, 1994).

The multiplicity of opioid receptor types involved in such peripheral effects has been questioned. Stein et al. (1989) showed that peripheral opioid receptors me-

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diating antinociception in inflammation possess distinguishable pharmacological characteristics resembling those of μ-, δ- and κ-opioid receptors. Since a novel κ-opioid receptor agonist which has limited access to the central nervous system (ICI 204448) has been synthesised, we investigated its antinociceptive effects after peripheral application in this model of mononeuropathy. Then, the putative abolition of these effects by a selective κ-opioid receptor antagonist, nor-binaltorphimine, was studied by using the vocalization thresholds to paw pressure.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Charles Rivers, France) weighing 175–200 g on arrival were used ($n = 66$). The animals were allowed to habituate to the colony room at least a week before the beginning of the experiments. Six were kept as control and had no induction of neuropathy. For the 60 others, after the surgical procedure (see below), they were kept in individual cages for 5 days and then housed 4–5 to a cage, with free access to food and drink. These conditions aimed to minimize discomfort to the animals and fulfilled the guidelines of the Ethical Standards of the International Association for the Study of Pain. For the same reasons the number of rats used was kept to a minimum.

2.2. Surgical procedure

The neuropathy was produced in the right hind paw according to the method described by Bennett and Xie (1988) and Attal et al. (1990). Under sodium pentobarbital anaesthesia (Nembutal, 50 mg/kg i.p.), four ligatures were tied loosely (catgut chrome 5/0), with about 1 mm spacing, around the common sciatic nerve. These animals develop abnormal pain-related behaviours, which, according to the taxonomic classification of the International Association for the Study of Pain (IASP) can be named 'allodynia' and 'hyperalgesia' to mechanical and thermal (hot and cold) stimulation of the nerve-injured paw. They also exhibit an abnormal position of this paw, possibly reflecting the reluctance of the animal to place it on the floor. The abnormal sensitivity is maximal between 2–3 weeks after the beginning of the constriction, with a recovery at 8–10 weeks. Some pain-related behaviours have also been observed for the contralateral limb, and are clearly related to the intensity of abnormal reactions observed for the nerve-injured paw (Attal et al., 1990).

2.3. Behavioural test

Nociceptive thresholds were determined by a modification of the Randall-Selitto method described previously (Refs. in Attal et al., 1990) where a constantly increasing pressure is applied to the hind paw until the rat squeaks. This response represents a more integrated nociceptive behaviour than the withdrawal of the paw and is especially sensitive to analgesic compounds particularly in this pain model (Attal et al., 1991; Desmeules et al., 1993). The Basile analgesimeter (Apelex) (tip diameter of the stylus: 1 mm) was used. Each animal was carefully handled and wrapped in a towel so that only the limbs and head were free. The hind paw was placed under the stylus, the probe being applied to the dorsal part of the paw between the 3rd and the 4th metatarsus in the sciatic nerve territory. The withdrawal reflex that usually occurs before vocalization was prevented by gently holding the rat's hind paw in position under the pusher until vocalization.

For each rat, a pre-operative threshold (mean of two consecutive stable thresholds expressed in grams) was determined. Neuropathic rats were used 15–21 days after surgery and had a mean weight of 340 g during testing sessions. At this time, as described previously, the abnormal pain behaviour is at a stable maximum (Bennett and Xie, 1988; Attal et al., 1990). Test sessions beginning at 09.30 h were carried out in a quiet room, away from the colony room. The rats were randomly assigned to groups of 5 for a series of tests and, for each rat, a preliminary or control threshold (mean of two consecutive stable thresholds expressed in grams) was determined before injection of the drugs. The experimenter was unaware of the drugs and doses used. Nociceptive pressure thresholds were then measured every 10 min after drug administration (to avoid nociceptor 'fatigue'), until they returned to baseline.

2.4. Drugs and test dosages

The following drugs were used: ICI 204448 ((*R*,*S*)-*N*-[2-(*N*-methyl-3,4-dichloro-phenylacetamido)-2-(3-carboxyphenyl)-ethyl]pyrrolidine hydrochloride)) (RBI – Research Biochemicals, Natick, MA, USA) a κ-opioid receptor agonist. Nor-binaltorphimine dihydrochloride (RBI – Research Biochemicals, Natick, MA, USA) a κ-opioid receptor antagonist. Saline (NaCl 0.9%).

In a first series of experiments, 5 groups of mononeuropathic rats were injected intraplantarly into the nerve injured paw (volume of 0.2 ml) with 20 ($n = 5$); 30 ($n = 6$); 40 ($n = 7$) and 50 ($n = 7$) μg of ICI 204448 or saline ($n = 6$). The doses of ICI 204448 were chosen with regard to previous experiments performed with carrageenin-induced hyperalgesic rats (unpublished experiments) where ICI 204448 (20 μg) had no effect while 40 μg produced a significant antinociceptive effect.

In a second series of experiments, 3 groups of neuropathic rats were tested to determine the effect of the

antagonist, nor-binaltorphimine, on the antinociceptive effect of ICI 204448. A first group of rats ($n = 7$) was injected i.pl. with 30 μg nor-binaltorphimine alone (volume of 0.1 ml). These experiments were performed to assess the capacity of the κ-opioid receptor antagonist, given alone, to alter the nociceptive threshold in mononeuropathic rats. The two other groups were injected with the most effective dose (40 μg) of ICI 204448 plus 20 μg ($n = 7$) or 30 μg ($n = 6$) nor-binaltorphimine. The doses of nor-binaltorphimine were less than those used i.pl. by Stein et al. (1989) (50 μg), in an attempt to reduce any possible systemic diffusion.

To assess whether the antinociceptive effects of ICI 204448 were mediated through a central site of action, an additional group of neuropathic rats was injected i.v. into a tail vein with 40 μg ICI 204448, in a volume of 0.2 ml ($n = 4$).

Finally, a control group of normal rats ($n = 6$) was injected i.pl. with 40 μg ICI 204448 (volume of 0.2 ml).

Drugs were freshly prepared in sterile physiological saline (0.9% NaCl). Each animal was used only once and received a single dose of drug or saline.

2.5. Statistical procedures

Vocalization thresholds are reported in grams. Data are expressed as means ± S.E.M. Values of the mean curves are expressed as percentages of the two control values measured just before drug administration. Statistical calculations using the values expressed in grams were done with a one-way analysis of variance (ANOVA) to compare the area under the curve (AUC) corresponding to the surface defined by the time/antinociception effect curve (expressed in g/min) and the corresponding baseline defined as the control vocalization threshold (in g). Post-hoc analysis used the Fisher's PLSD (paired-least significant differences) test. A paired Student's t-test was done to compare the vocalization threshold before and after surgery for each paw. Results were regarded as being significant when P values were less than 0.05.

3. Results

3.1. General results

Before nerve ligature, the vocalization threshold did not differ significantly between the hind paws, being 250 ± 9 g and 243 ± 10 g. As reported earlier (Attal et al., 1991; Desmeules et al., 1993), 2 weeks after the surgical procedure the mean threshold was markedly decreased to 135 ± 24.5 g (54%) for the nerve-injured paw ($t(30) = 14.41$, $P < 0.0001$, paired t-test) compared to the pre-operative value. Thus, we considered that this decreased threshold reflected mechanical allodynia (Attal et al., 1990). Although much less affected, the vocalization threshold of 225 ± 5 g (92.5%) was also significantly diminished for the contralateral paw ($t(30) = 2.72$, $P < 0.01$, paired t-test) compared to the pre-operative value.

After the injection of ICI 204448, no significant behavioural changes were noted in either group of rats, other than an increase in the vocalization threshold and recovery from the abnormal position of the nerve-injured paw.

3.2. Effects of the i.pl. injection of increasing doses of the κ-opioid receptor agonist

The effect of an i.pl. injection of ICI 204448 on the vocalization thresholds was investigated in four groups of mononeuropathic rats receiving 20, 30, 40 or 50 μg ICI 204448 into the nerve-injured paw (Table 1).

Nerve-injured paw (Fig. 1A)

In these animals, ICI 204448 had a tendency to increase the vocalization threshold, compared to saline,

Table 1
Maximal vocalization thresholds (g ± S.E.M.) for the nerve-injured paw in mononeuropathic rats, before and after i.pl. injection of saline, ICI 204448 (ICI), nor-binaltorphimine (Nor-BNI) 30 μg, or ICI 204448 + Nor-BNI

Treatment (μg)	Nerve-injured paw			Contralateral paw	
	Before injection	After injection	AUC	Before injection	After injection
Saline ($n = 6$)	125 ± 3	145 ± 14	319 ± 12	200 ± 5	200 ± 9
ICI 20 ($n = 5$)	135 ± 7	165 ± 15	349 ± 19	219 ± 7	213 ± 7
ICI 30 ($n = 6$)	132 ± 4	175 ± 18	349 ± 17	222.5 ± 14	220 ± 15
ICI 40 ($n = 7$)	139 ± 4	216 ± 6 [b]	419 ± 24 [d]	214 ± 12	240 ± 27
ICI 50 ($n = 7$)	133 ± 6	184 ± 187 [a]	379 ± 18 [c]	227 ± 16	240 ± 21
Nor-BNI 30 ($n = 7$)	182 ± 21	220 ± 45		307 ± 30	320 ± 51
ICI 40 + Nor-BNI 20 ($n = 7$)	128 ± 4	167 ± 15 [a]	334 ± 15 [e]	233 ± 13	234 ± 39
ICI 40 + Nor-BNI 30 ($n = 6$)	147 ± 13	148 ± 13	341 ± 33 [e]	257 ± 22	243 ± 30

Maximal vocalization thresholds for the contralateral paw before and after the same treatment. Since there was no significant effect for the contralateral paw, the mean AUC was not calculated. [a] $P < 0.05$; [b] $P < 0.001$ (paired t-test), comparisons of the maximal vocalization thresholds before and after drug injections for each paw. [c] $P < 0.05$; [d] $P < 0.001$ (Fisher's PLSD). AUC comparisons with saline. [e] $P < 0.05$ (Fisher's PLSD). AUC comparisons with ICI 204448 40 μg.

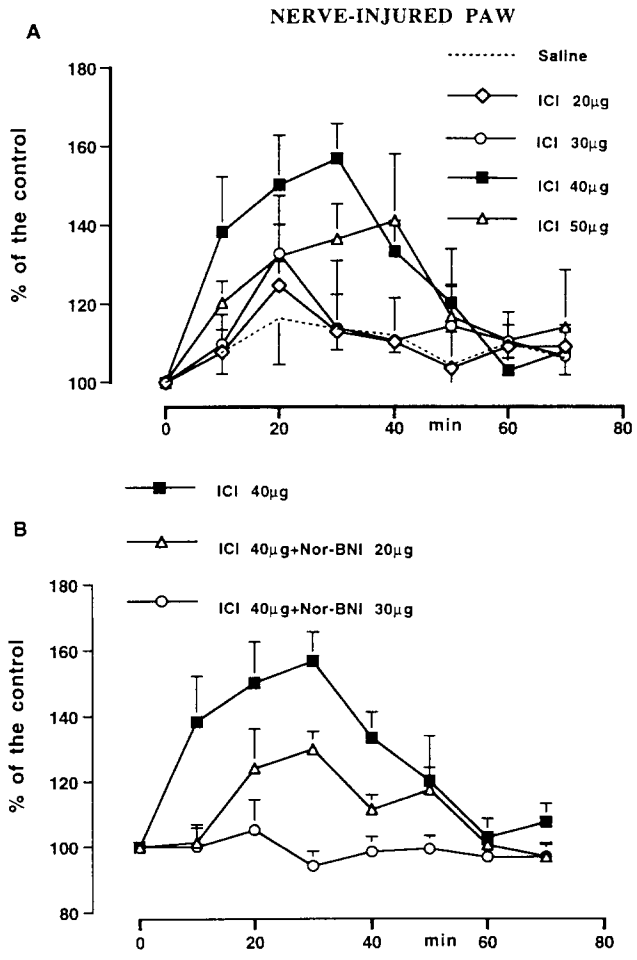

Fig. 1. A: Mean curves of the effects on the vocalization threshold to paw pressure of ICI 204448 20, 30, 40 and 50 μg or saline i.pl. in the nerve-injured paw. B: Mean curves of the effects on the vocalization threshold to paw pressure of ICI 204448 40 μg, ICI 204448 40 μg and nor-binaltorphimine (Nor-BNI) 20 μg or 30 μg i.pl. in the nerve-injured paw. Each value is expressed as a percentage of the mean baseline threshold + S.E.M.

with increasing doses (ANOVA, $F(4,26) = 4.216$; $P < 0.01$).

With 20 μg of ICI 204448, the maximal effect was observed 20 min after the injection. At this time, the mean vocalization threshold, expressed as a percentage of the control value, was 125 ± 15 (165 ± 15 g). For 30 μg the peak value at 20 min after the injection was 133 ± 14% of the control (175 ± 18 g). However, the effects of these two lower doses were not significantly different from saline, based on the AUC (post-hoc comparison, Fisher's PLSD, $P > 0.05$).

With the dose of 40 μg, the maximal effect reached at 30 min was 157 ± 9% of the control value (216 ± 6.5 g) and the overall effect lasted up to 60 min. These effects with ICI 204448 were significant compared to those of saline ($P < 0.05$, Fisher's PLSD comparison with saline for the AUC).

The highest dose of ICI 204448 (50 μg) had a maximal effect at 40 min, 141 ± 17% of the control value (184 ± 18 g), and comparison of the AUC revealed a significant difference compared to that of saline ($P < 0.05$, Fisher's PLSD comparison with saline for the AUC). Nevertheless, the effects of 50 μg and 40 μg of ICI 204448 were not significantly different ($P > 0.05$, Fisher's PLSD, comparison with 40 μg for the AUC).

Contralateral paw

No antinociceptive effect of ICI 204448 was observed for the contralateral paw. The maximal vocalization thresholds were: 99 ± 5, 110 ± 6 and 106 ± 5% of the control value, respectively for rats receiving 20, 30, 40 and 50 μg ICI 204448 i.pl.

3.3. Effects of the i.pl. injection of the κ-opioid receptor antagonist nor-binaltorphimine

The effects of an i.pl. injection of nor-binaltorphimine on the vocalization threshold were investigated in a group of mononeuropathic rats receiving nor-binaltorphimine 30 μg into the nerve-injured paw (Table 1).

In these animals, there was no significant effect on the nerve-injured paw (maximal vocalization threshold was 121 ± 6% of the control value, 220 ± 45 g) and the contralateral paw (103 ± 5%, 320 ± 51 g).

3.4. Antagonism by nor-binaltorphimine of the antinociceptive effect of ICI 204448 40 μg

The effect of an i.pl. injection of nor-binaltorphimine on the antinociception induced by ICI 204448 (40 μg) was investigated in two groups of mononeuropathic rats receiving nor-binaltorphimine 20 and 30 μg (Table 1).

Nerve-injured paw

In these animals, the antinociceptive effect of 40 μg of ICI 204448 was significantly antagonized by nor-binaltorphimine.

For rats receiving 20 μg nor-binaltorphimine, the maximal vocalization threshold was 130 ± 10% of the control at 30 min (167 ± 15 g), i.e., reduced by about 2-fold. The AUC was significantly different from that of the group receiving 40 μg of ICI 204448 alone ($P < 0.05$, Fisher's PLSD).

With 30 μg nor-binaltorphimine, the antinociceptive effect of ICI 204448 was almost abolished. The maximal effect reached at 20 min was 105 ± 9% (148 ± 13 g). The effect was significantly different to that of the group receiving 40 μg of ICI 204448 alone ($P < 0.05$, Fisher's PLSD comparison with 40 μg of ICI 204448 alone for the AUC).

Contralateral paw

No changes in the vocalization threshold after the co-injection of ICI 204448 and nor-binaltorphimine

were observed for the contralateral paw, compared with the effect of 40 μg of ICI 204448 alone.

3.5. Effects of i.v. injection of 40 μg ICI 204448

In the animals receiving an acute i.v. injection of ICI 204448 (40 μg), there was no significant effect on the nerve-injured paw (maximal vocalization threshold was 100 ± 8% of the control value, 135 ± 15 g) and the contralateral paw (100 ± 8%, 200 ± 7 g).

3.6. Effects of an i.pl. injection of 40 μg ICI 204448 in normal rats

No significant effect was elicited by 40 μg of ICI 204448 in normal rats, with the maximal vocalization threshold being 109 ± 10 and 100 ± 6% of the control for the injected and the contralateral paws respectively.

4. Discussion

In the present study, using a centrally integrated test, namely the vocalization threshold to paw pressure, a test which is highly sensitive to low doses of i.v. opioids (Refs. in Attal et al., 1990; Desmeules et al., 1993), we clearly established that the peripheral application of a κ-opioid receptor agonist which has limited access to the central nervous system is able to attenuate mechanical pain-related disorders caused by peripheral sciatic mononeuropathy in the rat.

In a previous study, using the same experimental model of clinical pain, Desmeules et al. (1993) demonstrated an impressive antinociceptive effect obtained with the κ-opioid receptor agonist, U 69593, with doses as low as 0.75 mg/kg i.v. in the same behavioral test. It has been suggested that a contributory mechanism could be a potential peripheral action of the κ-opioid receptor agonist on the nerve-injured paw. Therefore, we used a novel hydrophilic opioid, ICI 204448, which is well absorbed following subcutaneous administration and exhibits high selectivity towards κ-opioid receptors and low central nervous system penetration (Shaw et al., 1989). Furthermore, the demonstration of effects after local, but not after systemic, administration of equal doses of ICI 204448 (40 μg) provides evidence for a peripheral site of action.

In mononeuropathic rats, the lower doses of ICI 204448 (20 and 30 μg i.pl.) did not or only slightly alleviated the allodynia in response to mechanical stimuli. It seems unlikely that the poor effect of these low doses of ICI 204448 on the mechanical stimulus in the rat model of peripheral mononeuropathy could be due to the sensitivity of the test, since it has already been used to demonstrate potent antinociceptive activity of several drugs such as opioid and anti-depressant substances administered systemically in these animals (Refs. in Desmeules et al., 1993). By contrast, the higher doses (40 and 50 μg) produced good relief of mechanical allodynia. Although the vocalization threshold for the nerve-injured paw before any injection was lower in neuropathic rats, i.pl. injections of 40 μg ICI 204448 in the neuropathic animals restored the threshold to a 'normal' value (Table 1), and this effect lasted for 40 min. Although less marked than the effects of the systemic κ-opioid receptor agonist U-69,593 (0.75 mg/kg), these effects (mean maximal vocalization threshold increased by 60% of the control) (Fig. 1A) were roughly comparable in magnitude to those of morphine (0.6 mg/kg i.v.) in the same behavioral test in these animals (Attal et al., 1991). This effect was strictly restricted to the nerve-injured limb. An important other observation is the lack of side effects, such as the behavioural reaction described for κ-opioid receptor agonists injected systemically at high doses (Refs. in Desmeules et al., 1993). In addition, the antinociceptive effects of ICI 204448 (40 μg) were prevented by local application of the specific κ-opioid receptor antagonist nor-binaltorphimine, indicating the involvement of κ-opioid receptors. It should be noted that the antagonist alone (30 μg i.pl.) had no significant effect in this model, as shown previously with the same test after 1 mg/kg i.v. administration (Lee et al., 1994).

The mean curves obtained with 40 and 50 μg ICI 204448 were almost the same. Based on this, we considered it unjustified to test higher doses. The ceiling effect observed with ICI 204448 (40 and 50 μg) may be due to a saturation of the κ-opioid receptors present at the periphery. It is also possible that at these doses, a small amount of ICI 204448 could diffuse to the systemic circulation and, since the central nervous system penetration is not null (Shaw et al., 1989), cross the blood-brain barrier: a small amount of ICI 204448 acting centrally could counteract the antinociceptive peripheral effect by exerting a pronociceptive action, as suggested by other studies with κ-opioid receptor agonists and antagonists in different models of inflammatory and neuropathic pain (Ruda and Dubner, 1992; Refs. in Kayser, 1994).

In conclusion, it appears that the peripheral antinociceptive effects of κ-opioid receptor agonists can also be revealed in a model of persistent non-inflammatory pain where peripheral nerve fibres have been damaged by loose ligature. This effect appears to be limited by a ceiling effect.

Although inflammatory conditions are not obvious in the mononeuropathic model (Attal et al., 1990), the peripheral antinociceptive effect observed in the present pain model, and not in normal rats, suggests mechanisms resembling those proposed for inflammatory models (Stein et al., 1990), which may be linked to the

presence of activated macrophages around the injured nerve fibers (Bonetti et al.,1993; Sommer et al., 1993) and to the activation of κ-opioid receptors located on peripheral sensory neurones. It should be noted that alterations in endogenous opioid substances, such as dynorphin (an opioid peptide considered to be the endogenous ligand of κ-opioid receptors), and in κ-opioid binding sites have been observed in this model, at least in the spinal cord (Refs. in Desmeules et al., 1993).

It is thus possible that the peripheral action of systemic morphine in neuropathic rats is not only mediated via peripheral μ-opioid receptors as demonstrated using naloxone methiodide (Kayser, 1994), but also via peripheral κ-opioid receptors, as it is the case for the κ-opioid receptor agonist U-69,593.

Overall, our results obtained with an experimental model of painful peripheral neuropathy strongly suggest that κ-opioid receptor agonists (such as ICI 204448) injected locally may offer an additional therapeutic option for the alleviation of some clinical symptoms (such as mechano-allodynia) of painful peripheral neuropathic conditions and that further well conducted clinical psychophysical studies are needed to validate its use in selected patients.

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