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The antinociceptive effect of amisulpride in mice is mediated through opioid mechanisms

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Abstract

Antinociceptive effects of various neuroleptics in animal acute pain-models have been described, mediated trough different pathways including the opioid system. In this study, we assessed the antinociceptive effects of the atypical neuroleptic drug amisulpride, which acts as a selective blocker of dopamine D2 and D3 receptors. Furthermore, at low doses amisulpride has a selective preference for presynaptic dopamine autoreceptors, while at high doses it manifests a preferential action at post-synaptic dopamine receptors. We found amisulpride to be a potent antinociceptor agent in the mouse tail-flick assay, with an ED_{50} of 36.6 mg/kg. This effect was antagonized by naloxone ($P<0.05$), indicating an involvement of opioid mechanisms as mediators of the antinociceptive effect of amisulpride. β-Funaltrexamine (μ_1- and μ_2-opioid receptor antagonist), naloxonazine (selective μ_1-opioid receptor antagonist), naltrindole (selective δ-opioid receptor antagonist), Nor-binaltorphamine (κ_1-opioid receptor antagonist) reversed amisulpride antinociception at the same dose that they antagonized morphine's antinociceptive effect (all $P<0.005$). We found that the sensitivity of amisulpride-induced antinociception is mediated through selective involvement of all three opioid receptor subtypes. Based on previous studies with risperidone, clozapine and olanzapine we tend to attribute this global interaction with the opioid system to amisulpride's action at the dopamine D2 receptor sites.

Keywords: Amisulpride; Atypical neuroleptic; Opioid receptor sub-type; Antinociception; Tail-flick; Pain

1. Introduction

Although antinociceptive effects of various neuroleptics in animal pain-models have been described and assessed, neuroleptics as a group do not have a well-established place in the management of pain. The clinical use of the traditional neuroleptics for pain management was controversial at best and limited, due to the frequent complications of Parkinsonian and other side effects (Fields, 1989). These side effects are inevitable when treating psychosis, but outweigh benefit when treating pain, where other effective classes of drugs exist. Nevertheless, the clinical use of traditional neuroleptics, especially in combination with opiates or tricyclic antidepressants, is not uncommon (Dundee et al., 1963; Fields, 1989; Mersky and Hester, 1972; Taub, 1973; Zitman et al., 1991). Since the introduction of the newer generations of neuroleptics (both novel and atypical) with a reduced profile of side effects, the possible use of this class of medications in the management of pain is an issue to be addressed. In previous studies, we found that some new-generation antipsychotic agents augment differently opioid-induced antinociception, as in the case of the novel antipsychotic drug risperidone (Schreiber et al., 1997) and the atypical neuroleptic drugs clozapine and olanzapine (Schreiber et al., 1999).

Amisulpride is another new atypical antipsychotic agent, which chemically is part of a class of substituted benzamides. It acts as a selective blocker of dopamine D2 and D3 receptors, and has little affinity for other dopamine receptor subtypes (D1, D4 or D5) or other neurotransmitter receptors (serotonin, histamine, muscarinic, adrenergic) (Schoemaker et al., 1997). Its atypical profile depends on blockade of the mesolimbic dopaminergic tracts rather than nigrostriatal dopaminergic transmission and on a preferential blockade of dopamine D3 receptors in the limbic system (Castelli et al., 2001). At low doses it has a selective preference for presynaptic dopamine autoreceptors, thereby increasing dopaminergic transmission. This can explain its advantage in

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treating negative symptoms in schizophrenic patients (Lecrubier, 2002; Muller et al., 2002). However, at higher doses it blocks post-synaptic dopamine receptors, thus controlling the psychotic symptoms (Coulouvrat and Dondey-Nouvel, 1999; Perrault et al., 1997; Castelli et al., 2001; Leucht et al., 2002).

In a study evaluating the interaction of the dopamine D3 receptor agonist (±)-7-hydroxy-dipropylaminotetralin (7-OH-DPAT) with the opioid system, Cook et al. (1999) found it to induce a dose-dependent attenuation of the antinociceptive effects of morphine and dezocine. This finding suggests a possible modulation of antinociception of morphine mediated through the dopamine D3 receptor. Since amisulpride involves dopamine D3 receptor blockade, we hypothesized that it would exert an antinociceptive effect. Furthermore, since we found in our previous studies that different dopamine receptor subtypes mediate antinociception by different interactions with the opioid system (Schreiber et al., 1997, 1999), we assumed that assessing the antinociceptive properties of a drug that interacts both with dopamine D2 and D3 receptors (amisulpride) may shed some light on the different effects of dopamine D2 and D3 receptor subtypes on opioid regulation.

The aim of this present study was to evaluate whether amisulpride has an antinociceptive effect, and if so, is this effect mediated through opioid mechanisms.

2. Materials and methods

2.1. Subjects and surgery

Male ICR mice (age 5–6 weeks), from Tel-Aviv University colony (Tel-Aviv, Israel), weight 25–35 g were used. The mice were maintained on a 12-h light/12-h dark cycles with Purina rodent chow and water available ad libitum. Animals were housed five per cage in a room maintained at 22 + 0.5 °C until testing. Mice were used only once. Intrathecal (i.t.) injections were introduced by lumbar puncture (Hylden and Wilcox, 1980) all other injections were made subcutaneously.

2.2. Agents

Several agents were generously donated as follows: Amisulpride by Synthelabo Groupe (SylaChim, Mourenx, France), Morphine by TEVA (Jerusalem, Israel), naloxonazine by Dr. G.W. Pasternak from Memorial Sloan-Kettering Cancer Center, New York, USA, U50,488-H {*trans*-3,4-dchloro-*N*-methyl-*N*-[2-(1-pyrrolindinyl)-cyclohexyl]-benzeneacetamide} by Upjohn Pharmaceutics (West Sussex, England), [D-Pen^2,D-Pen^5] enkephalin (DPDPE), β-funaltrexamine, Naltrindole HCl, Nalorphine HCl, Naloxone HCl and Nor-binaltorphamine were obtained from the Research Technology Branch of NIDA. Amisulpride was dissolved in ethanol 70% and saline in 30:70 ratios. All other drugs were dissolved in saline.

2.3. Antinociception assessment

Mice were tested using the tail-flick apparatus (Ugo Basile). The latencies of tail withdraw from a focused light stimulus were measured electronically, using a photocell. Baseline latencies (1.0–3.5 s) were determined before experimental treatment for each mouse as the mean of two trials. Post-treatment latencies were determined after 30 min for each experiment and a maximal latency of 10 s was used to minimize tissue damage. Antinociception was defined quantitatively as doubling or more of baseline values for each mouse. For each dose at least 20 mice were checked and their scores were summarized, showing the percentage of animals, which became analgesic. Post-treatment latencies for all s.c. administrations were determined after 30 min and 15 min for the i.t. injected group.

2.4. Procedure

The study was conducted in three experiments.

2.4.1. Experiment 1

Groups of mice ($n = 20$) were injected subcutaneously with increasing doses of amisulpride (from 1 mg/kg to 100 mg/kg) to determine the effect of the drug in eliciting antinociception. Normal motor behavior was observed following amisulpride injection.

2.4.2. Experiment 2

The sensitivity of amisulpride to specific opioid receptor antagonists was examined. First we determined the effect of two doses of the non-selective opioid antagonist naloxone (1 and 10 mg/kg, s.c.) on high dose of amisulpride (75 mg/kg, s.c.). Both low and high doses of naloxone inhibited amisulpride antinociceptive effects, so we continued examining the effect of the specific opioid antagonists on amisulpride. Mice (at least 20 in each group) administrated with amisulpride were treated with one of the following drugs: β-funaltrexamine (μ_1- and μ_2-opioid receptor antagonist; 40 mg/kg, s.c.) or naloxonazine (μ_1-opioid receptor antagonist; 35 mg/kg, s.c.), 24 h before amisulpride challenge. Naltrindole (δ-opioid receptor antagonist) 20 mg/kg, s.c., Nor-binaltorphamine (κ-opioid receptor antagonist) 10 mg/kg, s.c. or with saline were injected at the same time with amisulpride. For comparison, each antagonist was tested against all the agonists in separate groups of mice. As expected, β-funaltrexamine and naloxonazine blocked only morphine's antinociception, Nor-binaltorphamine blocked U50, 488H antinociception and naltrindole blocked [D-Pen^2,D-Pen^5] enkephalin antinociception (data not shown). All the drugs and doses used in the present work were chosen in according to our pervious works.

2.4.3. Experiment 3

The sensitivity of amisulpride to specific opioid receptor agonists was examined. The action of amisulpride on selective opioid receptor subtype agonists was tested as follows: groups of mice ($n=20$) were given increasing doses of morphine (a μ-opioid receptor agonist), or with U50,488H (a κ_1-opioid receptor agonist), or with [D-Pen^2,D-Pen^5] enkephalin (a δ-opioid receptor agonist), or with nalorphine (a κ_3-opioid receptor agonist) with an inactive dose of amisulpride (1.0 mg/kg).

2.5. Statistic analysis

Dose–response curves were analyzed, using an SPSS computer program. This program maximizes the log-likelihood function to fit a parallel set of Gaussian normal sigmoid curves to the dose–response data. Single dose antagonist studies were analyzed using the Fisher exact test.

3. Results

3.1. Amisulpride antinociception

Amisulpride induced a dose-dependent antinociceptive effect following s.c. administration. The ED_{50} for mice in the tail-flick assay for amisulpride was 36.6 mg/kg (27.7; 47.8; 95% CL; Fig. 1).

3.2. Sensitivity of amisulpride antinociceptive effect to selective antagonists

High doses of amisulpride (75 mg/kg, which produced 90% antinociception) were used with low and with high doses of the non-specific opioid antagonist naloxone (1 mg/kg and 10 mg/kg). The antinociceptive effect of amisulpride was antagonized with both doses of naloxone to 30% ($P<0.05$; Fig. 2). These findings imply an opioid mechanism of action involved in amisulpride-induced antinociception.

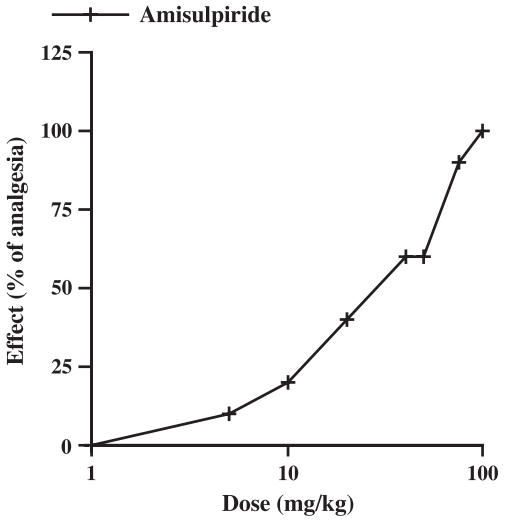

Fig. 1. Groups of mice ($n=20$) received a s.c. injection of amisulpride at the indicated dose and were tested in the tail-flick apparatus 30 min later. Dose–response curve of amisulpride antinociceptive effect.

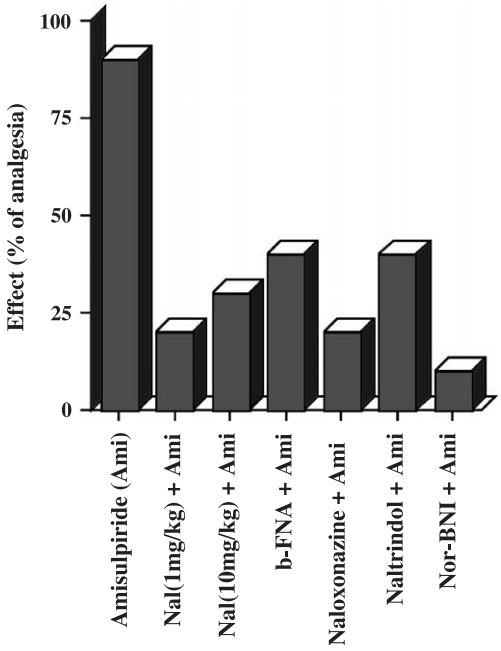

Fig. 2. Groups of mice ($n=20$) were treated with a high dose of amisulpride (75 mg/kg, s.c.) alone or were challenged in addition with naloxone (1.0 mg/kg, s.c.), β-funaltrexamine (β-FNA) (40 mg/kg, s.c.), naloxonazine (35 mg/kg, s.c.) naltrindole (20 mg/kg, s.c.) or Nor-binaltorphamine (Nor-BNI) (10 mg/kg, s.c.). Effects of opioid antagonists on amisulpride (ami) antinociceptive effect.

The possible involvement of μ-, δ-, and κ_1-opioid receptor subtypes in amisulpride antinociceptive effect was studied, using selective opioid antagonists (Fig. 2). We found that β-funaltrexamine (40 mg/kg; selective μ_1- and μ_2-opioid receptor antagonist) and naloxonazine (35 mg/kg; selective μ_1-opioid receptor antagonist), reversed amisulpride antinociception at the same dose that they antagonized morphine's antinociceptive effect ($P<0.005$). Naltrindole (10 mg/kg; selective δ-opioid receptor antagonist) reversed amisulpride induced antinociceptive effect at the same dose it antagonized δ-opioid receptor antinociception mediated by [D-Pen^2,D-Pen^5] enkephalin ($P<0.005$). Nor-binaltorphamine (10 mg/kg; selective k_1-opioid receptor antagonist) reversed amisulpride induced antinociceptive effect at the same dose that it antagonized κ_1-opioid receptor antinociception, mediated by U50,488H ($P<0.005$). The activity of each of the opioid receptor antagonists was confirmed with its prototypic agonists (data not shown). None of the opioid receptor antagonists mediated antinociception by themselves, nor did they change the baseline latencies of the pretreated animals. The described sensitivity of amisulpride antinociceptive effect to the selective opioid receptor antag-

Table 1
Amisulpride interaction (ED_{50}) with selective opioid agonists

Amisulpride	With vehicle	With amisulpride
Morphine (μ)	5.4 (3.7; 7.8)	3.4 (1.7; 5.2)
U50, 488 (κ_1)	1.9 (0.09; 3)	2.2 (0.2; 3.7)
Nalorphine (κ_3)	31.4 (13.4; 49.8)	31.4 (13.2; 50.13)
DPDPE (δ)	402.5 (251.5; 594.7)	163.7 (23.6; 317.9)

The numbers in parentheses are the with 95% confidence limits (CL) of the ED_{50}.
In each group 20 mice were used.

onists implies the selective involvement of all three opioid receptor subtypes, thus a global opioid mechanism.

3.3. Sensitivity of amisulpride antinociceptive effect to selective agonists

We injected the selective agonists of the opioid receptor subtypes with or without an inactive dose of amisulpride (1 mg/kg, s.c., Table 1). We found no significant shift in the dose–response curves, which indicate that there is no significant augmentation of the antinociceptive effects of opioid receptor agonists by amisulpride (Table 1).

4. Discussion

In previous studies, we have found the novel antipsychotic drug risperidone (a potent dopamine D2/5-HT_2 receptor antagonist) to interact with opioid receptors (mainly μ_1-, μ_2- and κ_1-opioid receptor subtypes), while the two atypical dibezo-benzodiazepines clozapine and olanzapine (both potent dopamine D3/D4/5-HT_2/α_2-adrenoreceptor and only weak dopamine D2-receptor antagonists) differed regarding their antinociceptive mechanisms. Clozapine induced antinociception mediated through opioid (μ_1-, μ_2-, κ_1-opioid receptor subtypes) and α_2-adrenoreceptors, while olanzapine induced nociception mediated through α_2-adrenoreceptors, with only a weak involvement of the opioid system. Since amisulpride blocks both dopamine D2/D3 receptor subtypes, we thought it would be interesting to assess the direct influence of dopamine D3 receptor subtype in antinociception and analgesia, and compare it to our previous findings with risperidone, clozapine and olanzapine. And indeed, in the present study we have found that amisulpride manifests an antinociceptive effect in a dose-dependent manner, mediated through global opioid mechanisms. However, the present results show that this antinociceptive effect is blocked by naloxone and by various selective opioid receptor subtype antagonists with no specific augmentation of any opioid receptor subtype agonist when given with sub-threshold doses of amisulpride. These results may support a global involvement of the opioid system in the antinociceptive effect of amisulpride. Contemplating these findings together with those of our previous ones, we stipulate that the opioid properties of amisulpride are mediated by its dopamine D2 receptor subtype properties (and not its dopamine D3 receptor subtype involvement).

The interactions of the dopaminergic system with the opioid system in modulation of pain is not so simple, although it is clear that both systems interact with each other in more than one way. Activation of dopamine that lowered opioid analgesia and enhanced analgesia with dopamine receptor antagonists was found in several studies (Tulunay et al., 1975; Rodgers, 1977). Recent studies also show involvement of the dopaminergic system in mechanisms of antinociception. In some studies, dopamine receptors agonists facilitated analgesic response (Michael-Titus et al., 1990; Morgan and Franklin, 1991). Carta et al. (1999) found that the dopamine D2 receptor antagonist sulpiride suppresses the antinociceptive effects of Δ^9-Tetrahydrocannabinol using hot plate and tail-flick tests in rats. In contrast, the antinociceptive effect of morphine in diabetic mice (but not that in non-diabetic mice) was significantly enhanced following pretreatment with sulpiride (Kamei and Saitoh, 1996). Moreover, sulpiride has been also found to antagonize the dopamine-induced antinociceptive effect in rats treated with dopamine injection to the lumbar subarachnoid space (Liu et al., 1992). King et al. (2001) supported the traditional studies that suggested anti opioid activity of the dopamine D2 receptor subtype by using dopamine D2 receptor subtype knock-out mice.

Other studies show similar findings about the relation between antinociception and dopamine (Altier and Stewart, 1998). In effort to specify which dopamine receptor subtype is involved in analgesia and antinociception, many researchers used dopamine D1- and D2-receptor subtype agonists or antagonists. The dopamine D1 receptor subtype in knock-out mice was found not to be involved in antinociceptive effect of κ-opioid receptors in preweanling mice using the tail-flick assay (Karper et al., 2000). Pretreatment with either the selective dopamine D1 receptor antagonist SCH 23390 or the dopamine D2 receptor antagonist (−)-sulpiride converted the hyperalgesia produced by the dopamine D1 receptor agonist into an antinociceptive response, whereas the effect of the dopamine D2 receptor agonist was significantly antagonized. Sulpiride also increased antinociception induced by the κ-opioid receptor agonist U50,488H (Rooney and Sewell, 1989). These findings support the relation between dopamine D2 receptor antagonists and antinociception effect.

Clozapine, a potent dopamine D4 receptor antagonist comparing to its weak dopamine D2 receptor blockade (as well as serotonergic, muscarinic, histaminergic and alpha adrenergic involvements) has been found to exert an opioid-mediated antinociceptive effect (Schreiber et al., 1999). The information about newer atypical neuroleptics does not address the possible pain regulation. In a study that compare the efficacy of Serotonin Selective Reuptake Inhibitors and amisulpride in burning mouth syndrome, the data suggest that both amisulpride and Serotonin Selective Reuptake Inhibitors may be effective treatments (Maina et al.,

2002). Further studies are needed in order to assess the relations of new atypical neuroleptics via their possible opioid involvement to antinociception and analgesia, and the possible use of this class of drugs in the pain clinic.

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