BASIC RESEARCH

Effect of glibenclamide on antinociceptive effects of antidepressants of different classes

Valiollah Hajhashemi,[I,II] Bahareh Amin[II]

[I] Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.
[II] Department of Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

OBJECTIVES: The purpose of this work was to determine whether the intraperitoneal administration of glibenclamide as a K_{ATP} channel blocker could have an effect on the antinociceptive effects of antidepressants with different mechanisms of action.

METHODS: Three antidepressant drugs, amitriptyline as a dual-action, nonselective inhibitor of noradrenaline and a serotonin reuptake inhibitor, fluvoxamine as a selective serotonin reuptake inhibitor and maprotiline as a selective noradrenaline reuptake inhibitor, were selected, and the effect of glibenclamide on their antinociceptive activities was assessed in male Swiss mice (25-30 g) using a formalin test.

DISCUSSION: None of the drugs affected acute nociceptive responses during the first phase. Amitriptyline (5, 10 mg/kg), maprotiline (10, 20 mg/kg) and fluvoxamine (20 and 30 mg/kg) effectively inhibited pain induction caused by the second phase of the formalin test. Glibenclamide (5 mg/kg) alone did not alter licking behaviors based on a comparison with the control group. However, the pretreatment of animals with glibenclamide (10 and 15 mg/kg) partially reversed the antinociceptive effects of fluvoxamine but not those of maprotiline. In addition, the highest dose of glibenclamide (15 mg/kg) partially prevented the analgesic effect of amitriptyline.

CONCLUSION: Therefore, it seems that adenosine triphosphate-dependent potassium channels have a major role in the analgesic activity of amitriptyline and fluvoxamine.

KEYWORDS: Antidepressants; Antinociceptive; ATP dependent K^+ channels.

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E-mail: vhajhashemi@gmail.com

Tel.: 0098 311 7922630

INTRODUCTION

After an annoyance by a noxious stimulus, peripheral nociceptor fibers translate sensory information into the dorsal horn of spinal cord and then through central ascending fibers to brainstem, thalamus and cerebral cortex for processing and perception of pain. With potentiation of descending inhibitory outputs including direct pathways [via gamma aminobutyric acid (GABA), opioids and cannabinoids] and indirect pathways (via 5-Hydroxytriptamine, noradrenaline, and acetylcholine) and modulation of sodium and calcium channels, we can overcome and relieve pain syndromes.[1,2]

For many years antidepressants have been shown to possess analgesic activity in different kinds of pain models in animals. They are frequently used in chronic pain conditions including diabetic neuropathy, post-herpetic neuralgia, headaches, cancer, chronic back pain and phantom limb pain and their effects are unrelated to their antidepressant effects because the doses used for analgesic actions are lower than antidepressant effects.[3-6] These drugs can increase noradrenaline (NA) and/or 5-hydroxytriptamine (5-HT) concentration in the extracellular space via inhibition of reuptake by blocking their transporters. Antidepressants are classified into different classes, some of them nonselectively block the reuptake of NA and 5-HT including tricyclic antidepressants such as amitriptyline, clomipramine and newer ones such as duloxetine and milnacipran. Some of them are selective for NA (SNRIs) such as maprotiline, or selective for 5-HT (SSRIs) such as fluoxetine, sertraline and fluvoxamine.[5-7]

However it has been shown that blockade of neurotransmitter reuptake is not the only mechanism involved in and there are additional central and peripheral mechanisms that contribute to either their antinociceptive actions or even adverse effects of these drugs. Inhibition of adenosine reuptake is one of the peripheral mechanisms in antinociceptive effects of these drugs.[8,9] Other potential mechanisms are inhibiting the functions of several other receptors including 5-HT_{2C}, 5-HT_3 and nicotinic acetylcholine receptors and affecting ion channels, by blocking the activity of voltage-gated Na^+ and Ca^{2+} channels or opening of K^+channels.[10-15] It has been shown that antidepressant drugs can potentiate analgesic effect of opioids by increasing the level of opioids.[16,17]

Modulation of microglia activation is another mechanism counteracting with excitotoxicity induced by glutamate that has an important role in pain signaling.[18] In a recent study it has been indicated that amitriptyline increases reuptake of glutamate through its transporters resulting in prevention of *N*-methyl-D-aspartate (NMDA) induced excitotoxicity.[19-20]

Neural K^+ channels are typically classified to four classes according to their structure, and specific agonists and antagonists that consist of voltage-gated (K_v), calcium-activated (K_{Ca}), inward rectifier (K_{ir}) and two-pore (K_{2P}) K^+ channels. It has been established that central K^+ channels especially ATP-sensitive K^+ channels (K_{ATP}) belonging to the K_{ir} family are involved in the perception of pain.[21] Central administration of K^+channel openers, such as diazoxide, minoxidil, lemakalim and cromakalim, produced antinociception and potentiated analgesic effect produced by opioid and α_2-adrenoceptor agonists.[22,23] Furthermore, central administration of ATP dependent K^+channel blocker, gliquidone, a sulfonylurea, reversed the analgesic action of amitriptyline and clomipramine in a hot plate test.[15] In another study intracerebro-ventricular injection of antisense oligonucleotide produced a dose-dependent inhibition of clomipramine and amitriptyline antinociception.[24] It seems that activation of G-protein coupled receptors by agonists such as α_2-adrenoceptors, opioids, $GABA_B$, muscarinic M_2, adenosine A_1, serotonin 5-HT_{1A} and cannabinoid receptors and some nonsteroidal anti-inflammatory drugs (NSAIDs) can activate these K^+ channels.[21] The purpose of this work was to examine if intraperitoneal administration of a K_{ATP} blocker, glibenclamide, one of the most important drugs in treatment of diabetic patients could also affect antinociptive effects of antidepressants with different mechanisms of action with respect to reuptake inhibition of 5-HT or NA. For this purpose, the effect of pretreatment with glibenclamide on the analgesic effect of amitriptyline as a dual action, non selective inhibitor of noradrenaline and serotonin reuptake, fluvoxamine as a selective serotonin reuptake inhibitor (SSRI) and maprotiline as a selective noradrenaline reuptake inhibitor (SNRI) was assessed in mice.

MATHERIAL AND METHODS

Animals

Male NMRI mice (Pasteur Institute, Tehran, Iran), weight range between 25-30 g were used. They were housed in cages in groups of six at 21 $\pm$ 2℃ in a 12 h light-dark cycle. Tap water and standard food pellets were available *ad libitum*. In order to minimize circadian rhythm influence, all experiments were conducted between 08:00 and 13:00 h, in a noise-free room with controlled lighting. Minimum of six mice were used for each treatment group. All procedures were approved by the Ethical Committee of the Isfahan University of Medical Sciences, and conducted in accordance with the internationally accepted principles for laboratory animal use and care. At the end of the experiments animals sacrificed in an ether chamber. Thirty minutes before testing, animals were placed in observation plexiglas box for accommodation.

Drugs

The following drugs were used: Amitriptyline hydrochloride (Iran Daru Pharmaceutical Co., Tehran, Iran), maprotiline (Razk Pharmaceutical Co., Tehran, Iran), fluvoxamine (Abidi Pharmaceutical Co., Tehran, Iran) and glibenclamide (Sigma, St Louis, Mo, USA). All drugs except glibenclamide were dissolved in normal saline 0.9% (w/v) and glibenclamide was suspended in water using 0.05% (w/v) carboxymethylcellulose. Glibenclamide was injected 15 min before antidepressant drugs and antidepressants administered 30 min prior to foot pad injection of formalin.

Measurement of antinociceptive activity

For evaluating antinociceptive effect of drugs we used formalin test in mice. This is a widely used assay for analgesic agents, which allow explaining the effects of analgesic agents used in clinical practice and is consumed a method for clinical inflammatory pain.[25] This method consists of two phase, phase I (acute phase, 0-5 min) and phase II (tonic phase, 20-40 min). Irritation in phase I produced by stimulation of nociceptors during the first 5 min after intra-plantar formalin injection and phase II is due to central sensitization inflammatory pathways. Twenty micro-liter of diluted formalin (2.5%) was injected subcutaneously into the dorsal surface of the right hind paw. Animals were then returned to the chambers, and measurement of the time spent for paw licking was performed during 0-5 (phase I) and 20-40 min (phase II) periods after formalin injection by a skilled observer blinded to drug treatments. All drugs were administered via intraperitoneal injections (i.p.).

Statistical analysis

The data were expressed as mean $\pm$ SEM and analyzed by one-way analysis of variance (ANOVA) followed by Scheffe post-hoc test, using SPSS 15.0 software. $P\ value < 0.05$ was considered significant.

RESULTS

Amitriptyline, fluvoxamine and maprotiline did not produce any considerable antinociception in the first phase of formalin test (data not showed). As shown in fig. 1 amitriptyline at doses of 5, 10 mg/kg significantly ($P<0.001$) inhibited paw licking behavior of second phase of formalin test and glibenclamide (15 mg/kg) partially reversed this effect. Antinociceptive effect of fluvoxamine on phase 2 has been shown in fig. 2. This drug showed significant analgesia in phase two, so that the percentage inhibition of paw licking behavior at doses of 20 and 30 mg/kg were 97% and 99% respectively. Glibenclamide alone had not any effect on formalin induced pain response, but at doses of 10 mg/kg and 15 mg/kg could partially reserve analgesic effect of 20 mg/kg fluvoxamine. Fig. 3 shows the effect of maprotiline on chronic phase of formalin. Maprotiline produced a considerable analgesic effect in phase two of formalin test at doses of 10 and 20 mg/kg. However, glibenclamide could not produce any significant inhibition of maprotiline-induced analgesia.

DISCUSSION

In this work, analgesic effects of three antidepressants belonging to different classes was examined in formalin test which is one of the most common animal tests for evaluation of analgesic drugs. In this study none of the drugs could significantly alter licking induced by formalin in phase one compared to control group, but all three antidepressants effectively attenuated pain behavior in

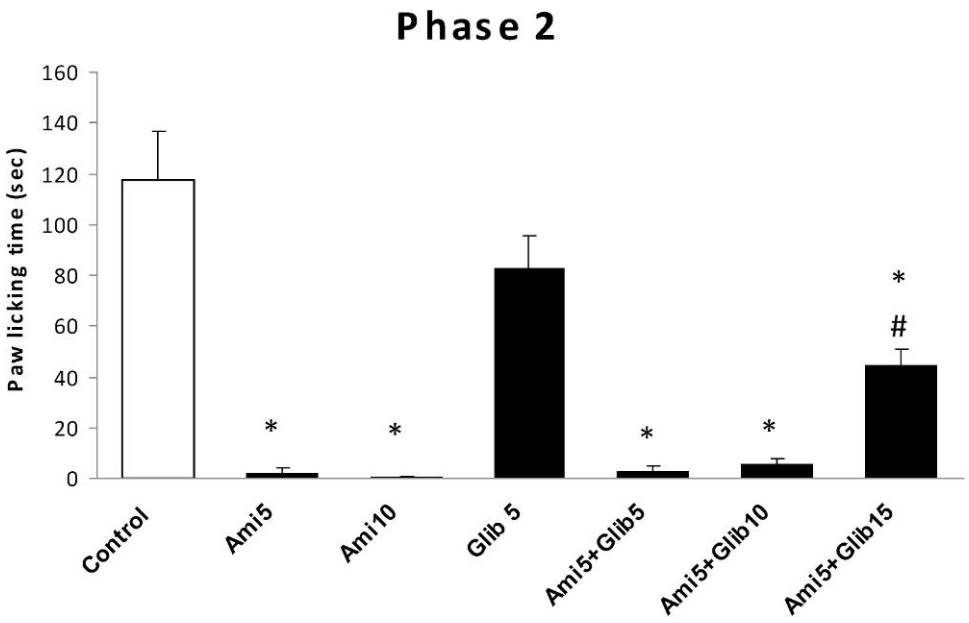

Figure 1 - The antinociceptive effect of i.p. administration of amitriptyline and amitriptyline plus glibenclamide on licking behavior during phase 2 of formalin test. Bars are mean ± SEM for six animals. * means significantly different from control group ($p<0.001$) and # means significantly different from Ami5 group ($p<0.05$).

phase two of formalin test that is in agreement with previous studies.[3,5] As indicated, phase 2 of formalin test is due to central sensitization of inflammatory pathways. This kind of sensitization is a key mechanism in inducing chronic pain.[26] These data support the beneficial effect of antidepressants in chronic pain conditions. Glibenclamide neither in phase 1 nor in phase 2 could attenuate pain behavior caused by formalin and this is in agreement with previous studies that showed the sulphonylurea drugs such as glibenclamide did not cause hyperalgesia or antinociception when administered alone.[15,22] As it was mentioned in introduction, activation of G-protein coupled receptors by agonists such as α_2-adrenoceptors, opioids, $GABA_B$, muscarinic M_2, adenosine A_1, serotonin 5-HT_{1A} and cannabinoid receptors and some nonsteroidal anti-inflammatory drugs (NSAIDs) can activate K^+ channels and this mechanism to some extent explains their antinociceptive effects.[21] In the absence of analgesic drugs there is no activation of these

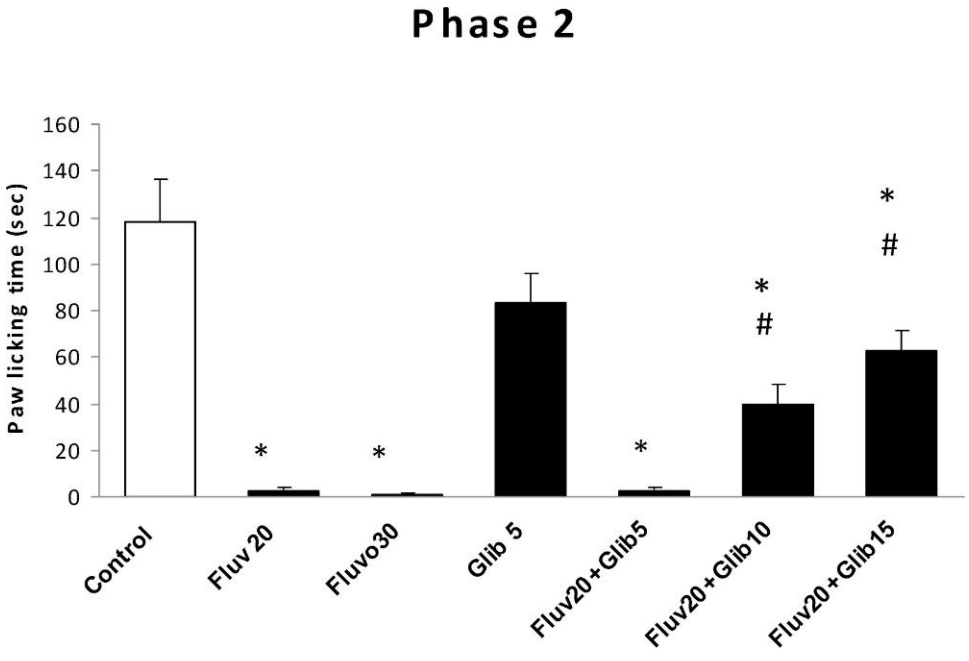

Figure 2 - The antinociceptive effect of i.p. administration of fluvoxamine and fluvoxamine plus glibenclamide on licking behavior during phase 2 of formalin test. Bars are mean ± SEM for six animals. * means significantly different from control group ($p<0.001$) and # means significantly different from Fluv20 group ($p<0.05$).

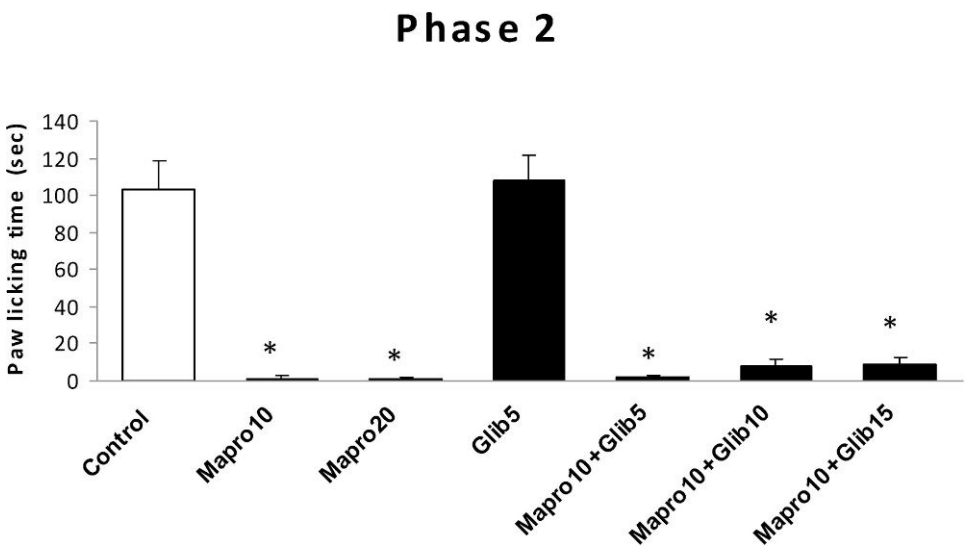

Figure 3 - The antinociceptive effect of i.p. administration of maprotiline and maprotiline plus glibenclamide on licking behavior during phase 2 of formalin test. Bars are mean ± SEM for six animals. * significantly different from control group ($p<0.001$).

channels and therefore we do not expect K^+ channel blockers alone to exert hyperalgesis or analgesia. As many other experimental works, our work has some limitations. One of them is the use of only one dose (5 mg/kg) of glibenclamide alone for a control group and it would be better to use doses of 10 and 15 mg/kg of this drug.

In the present study, glibenclamide could diminish antinociceptive effect produced by amitriptyline a non selective inhibitor of noradrenaline-serotonin and fluvoxamine a selective serotonin reuptake inhibitor and it shows that ATP-dependent K^+ channels have some role in antinociceptive activity of these antidepressants. Meanwhile inhibition of analgesic effect of fluvoxamine was greater than that of amitriptyline. Glibenclamide even at the highest dose could not inhibit maprotiline-induced analgesia which shows that ATP-dependent K^+ channels do not mediate its analgesia. Although Casis et al. showed that maprotiline block delayed rectifier potassium current in ventricular myocytes, but further studies are needed to clarify the exact role of these currents in neuronal pathways affected by maprotiline.[27]

It has been shown that different mechanisms are responsible for analgesic effects observed with antidepressants. The main action seems to be an increase in content of noradrenalin and serotonin in synaptic space followed by increased activity on their receptors. Serotonin is a neurotransmitter with complex actions that causes both inhibition and augmentation of pain perception depending on the subtype of receptor which is stimulated. Until now different subtypes of 5-HT receptors have been known, including 5-HT_1 to 5-HT_7. Earlier investigations have indicated that stimulation of 5-HT_{1A} receptor, a G_i-protein-coupled receptor which is located throughout of central nervous system (CNS) especially dorsal raphe nucleus contributes in antinociception by descending pathways.[28-31] Stimulation of this receptor by 5-HT leads to increase in potassium current and finally decrease in intracellular cAMP content.[28] Robels and coworkers indicated involvement of ATP sensitive potassium channels in analgesic action of 5-HT_{1A} agonists.[32] With respect to our results and previous data,[15] we can suppose that stimulation of 5-HT_{1A} receptors by elevated 5-HT in synaptic space by antidepressants acting on reuptake of 5-HT, might partially participate in analgesic activity of these drugs through affecting on ATP dependent potassium channels. Therefore opening of these channels have a major role in antinociceptive effects of them. Further work by applying $5HT_{1A}$ agonists and antagonists could help to have a better understanding of these pathways in antinociceptive effect of antidepressants.

It is also concluded that ATP-sensitive potassium channels may have no role in antinociceptive effect of maprotiline as a selective NA reuptake inhibitor because it has not been affected by a potassium channel blocker glibenclamide.

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