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Antinociceptive effects of gabapentin & its mechanism of action in experimental animal studies

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***Background & objectives*: Several studies have shown the possible analgesic effects of gabapentin, widely used as an antiepileptic. Thus, clinical studies have been carried out especially for neuropathic syndroms. This study was undertaken to investigate experimentally whether gabapentin has analgesic effects in mice and rats.**

***Methods*: The mice were divided into 10 groups (n=7) with various treatments to assess central and peripheral antinociceptive activity of gabapentin. Hot plate, tail clip and tail flick tests were applied for the investigation of central antinociceptive activity and the writhing test was applied for the investigation of peripheral antinociceptive activity. In addition, we also evaluated the levels of PGE_2 and nNOS on perfused hippocampus slices of rats.**

***Results*: Gabapentin showed a peripheral antinociceptive effect at all doses and a central antinociceptive effect at 30mg/kg dose. While the L-NAME and cyproheptadine changed the central and peripheral effects of gabapentin, naloxone did not change these effects. *In vitro* studies showed that gabapentin significantly increased nNOS level. PGE_2 and nNOS were found to have an important role in the antinociceptive effects of gabapentin at all doses and its combinations with L-NAME, cyproheptadine, indomethacine, and naloxone. As expected, PGE_2 levels decreased in all groups, while nNOS levels increased, which is believed to be an adaptation mechanism.**

***Interpretation & conclusions*: Our findings indicate that arachidonate, nitrergic and serotonergic systems play an important role in the antinociceptive activity of gabapentin except for the opioidergic system. Additionally, this effect occured centrally and peripherally. These effects were also mediated by nNOS and PGE2.**

Key words Antinociceptive effect - gabapentin - mice - nNOS - PGE_2 - rat hippocampus slices

Pain is a common situation and is one of the most frequently observed symptoms of different pathologies. Management of pain is particularly challenging and many patients suffering from chronic and subacut pain need a multidisiplinary approach. The principal targets of effective pain control are to ameliorate nociception, to reduce threshold of pain sensation, and to improve quality of life. Gabapentin has been used extensively for many years in the treatment of epilepsy. However, it has now been shown to have pain-relieving properties especially for the relief of pain caused by nerve damage. Gabapentin [1-(aminomethyl)- cyclohexane

acetic acid] a structural analogue of GABA (gama-aminobutyric acid) is used as an anticonvulsant and analgesic drug[1]. Its mechanism and site of action are not yet clearly understood. Several neurotransmitters such as GABA, adenosine, serotonine, noradrenaline and acetylcholine have been reported to modulate the pain mechanism at the central nervous system[2].

The non epileptic use of gabapentin is in neuropathic pain, and its efficacy has been demonstrated in treatment of postherpetic neuralgia, diabetic neuropathy, trigeminal neuralgia, radiculopathies, chemotheraphy and alcohol polineuropathies[3], fantom pains, acute artritis and migrain prophylaxis. Gabapentin has been shown to attenuate nocioceptive behaviour in acute arthritis model in rats[4] and has shown positive results in non-neuropathic headache and neck pain[5].

In many experimental pain model studies, it has been observed that gabapentin eliminates hyperalgesia. Although it has some side effects, which generally disappear or subside within two weeks without interrupting the treatment[4]. Intrathechal change has not been observed in haemodynamic studies and no change has been observed after intraperitoneal administration. It has been reported that gabapentin slightly increases the systolic and diastolic blood pressure on intra-cerebroventricüler administration[5-7]. Studies have primarily been conducted on its effects on $GABA_B$ receptors[4].

Though gabapentin is being used in many neuropathies originating from acute, chronic, central, and peripheral pains, the mechanisms of its action have not yet been explained in detail. Studies have shown that it blocks voltage dependant Na^+ and Ca^{++} channels, and opens K^+ channels[8]. Considering effective pathways in pain, the roles of opioidergic, arachidonergic, and serotonergic pathways cannot be denied.

In this study we aimed to examine the antinociceptive properties of gabapentin at spinal and supraspinal levels in experimental animals, and mechanisms involved in these effects.

Material & Methods

This study was conducted in the department of Pharmacology, Eskisehir Osmangazi University, Eskisehir, Turkey. The study protocol was approved by the Local Ethics Committee for Animal Experimentation of the University.

In vivo antinociceptive activity of gabapentin: Male Swiss Albino mice weighing 35±5 g were used. The experiment was carried out under laboratory settings at room temperature of 21^0C and alternating light and dark periods of 12 h each. The mice received identical diet and water *ad libitum*. The mice were divided into 10 groups (7 mice/group) as follows:

Group 1- Control (saline) i.p.; Group 2- gabapentin 10 mg/kg i.p.; Group 3- gabapentin 30 mg/kg i.p.; Group 4- gabapentin 100 mg/kg i.p.; Group 5- cyproheptadine 2 µg/kg i.p.; Group 6- gabapentin 30 mg/kg + cyproheptadine 2 µg/kg i.p.; Group 7- (L-NAME) 100 mg/kg i.p.; Group 8- gabapentin 30 mg/kg +L-NAME 100 mg/kg i.p.; Group 9- gabapentin 30 mg/kg+L-arginine 100 mg/kg i.p.; and Group 10- gabapentin 30 mg/kg+naloxone 1 mg/kg i.p.

Central effects were examined through hot plate[9], tail clip[10], and tail flick[11] tests one hour after the mice were given the medication in groups intraperitoneally[12]. For the peripheral effects the writhing test was used[13].

The central antinociceptive effect was calculated using the following formula[14]:

%MPE (Maximal potent effect) = Post-drug – pre-drug / cut-off-pre-drug X 100

The cut-off values were considered to be 30 sec.

Assessment of neuronal nitric oxide synthase (nNOS) in the slices of rat hippocampus: Male Sprague Dawley rats weighing 250±20 g were used. Immediately after ether anaesthesia, the brain tissue was rapidly excised and placed into ice-cold Kreb's solution (NaCI, 118.3 mmol/l; KCl, 4.7 mmol/l; $MgSO_4.7H_2O$, 1.2 mmol/l; KH_2PO_4, 1.2 mmol/l; glucose, 11.1 mmol/l; $NaHCO_3$, 25 mmol/l; $CaCI_2$, 2.5 mmol/l). The hippocampus was isolated, and sectioned into 0.6 µm thick slices using a chopper. The slices were washed at 37^0C for 1 h at every 15 min, and allowed to stabilise in special chambers prepared in-house in an isolated organ bath. The hippocampal slices were incubated for 1 h in the presence of Kreb's solution in the perfusion system. When using two agents, the second agent was given 30 min after the first one. During the experiment, the incubation system was filled with a mixture of 95 per cent O_2 and 5 per cent CO_2 at each phase. The resultant perfusates were used in the assessment of nNOS activity. The hippocampus slices were also used for protein measurements[14] after being homogenized.

nNOS assessment: nNOS was measured spectrophotometrically in the perfusates using a commercial ELISA kit (Cayman Chemical Co., Ann Arbor, Michigan, USA). Spectrophotometric

evaluations were made at wavelenght of 540 nm using the Multiscan Ex-Elisa Reader (Biotech Lab. Equipment, Chicago, USA). The results given are in µM.

Prostaglandin E_2 (PGE_2) assessment: Spectrophotometric measurements were performed in the specimens using an ELISA kit (Cayman Chemical Co., Ann Arbor, Michigan, USA). Spectrophotometric evalutions were made at wavelenght of 540 nm using the Multiscan Ex Elisa Reader. The results are given in pg/ml.

Group 1- control (SF); group 2- gabapentin $5x10^{-3}$ M; group 3- gabapentin $4x10^{-3}$ M; group 4- gabapentin $3x10^{-3}$ M; group 5- cyproheptadine $5x10^{-3}$ M; group 6- cyproheptadine $4x10^{-3}$ M; group 7- cyproheptadine $3x10^{-3}$ M; group 8- gabapentin $4x10^{-3}$ M + cyproheptadine $4x10^{-3}$ M; group 9- L-NAME $5x10^{-3}$ M; group 10- L-NAME $4x10^{-3}$ M; group 11- L-NAME $3x10^{-3}$ M; group 12- gabapentin $4x10^{-3}$M +L-NAME $4x10^{-3}$ M; group 13- indomethacine $5x10^{-3}$ M; group 14- indomethacine $4x10^{-3}$ M; group 15- indomethacine $3x10^{-3}$ M; group 16- gabapentin $4x10^{-3}$ M + indomethacine $4x10^{-3}$ M; group 17- gabapentin $4x10^{-3}$ M + naloxone $4x10^{-3}$ M.

All chemical materials in these groups were dissolved in saline.

Statistical analysis: Kruskal-Wallis test was used for tail clip, tail flick to radiant heat and hot plate tests (pain behaviour tests for central antinociceptive activity). One way ANOVA was used for writhing test and evaluation of PGE_2 and nNOS.

Results

In vivo antinociceptive activity in mice: Gabapentin exhibited antinociceptive effect through peripheral paths at all doses (Fig. 1). Further, 30 mg/kg gabapentin revealed a central antinociceptive effect at spinal and supraspinal level (Fig. 2). At 2 µg/kg dose cyproheptadine showed peripheral antinociceptive effect (Fig. 3) , but revealed no central antinociceptive effect (Fig. 4).

Cyproheptadine enabled 30 mg/kg gabapentin to show a central antinociceptive effect (Fig. 4). However, it eliminated its peripheral antinociceptive effect (Fig. 2). Naloxone did not change the central and peripheral effects of gabapentin (Figs 2 and 4). L-arginine reduced the peripheral antinociceptive effect of 30 mg/kg gabapentin (Fig. 2), but did not affect its central effect (Fig. 4). L NAME used in conjunction

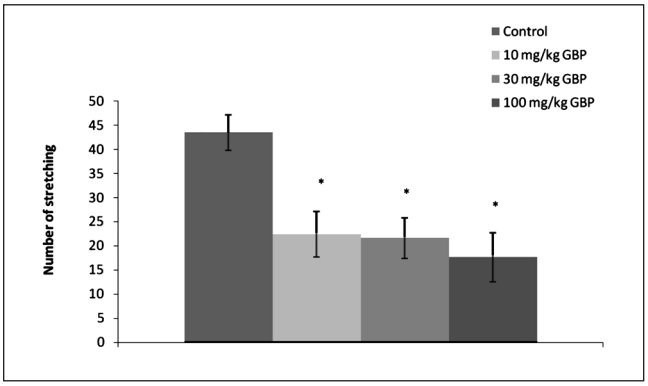

Fig. 1. The peripheral antinociceptive activity with different doses of gabapentin (GBP). *P<0.05 compared to control group. Values are mean ± SEM (n=7).

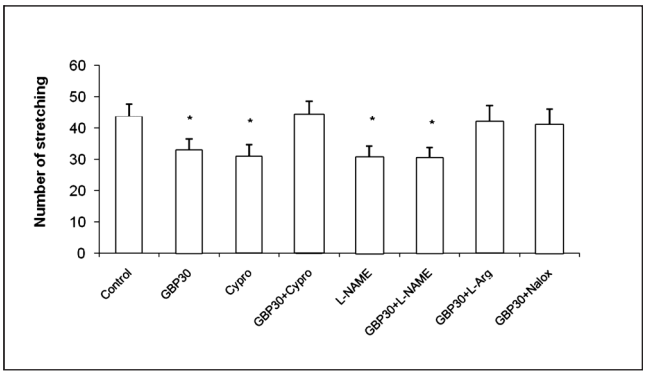

Fig. 2. The peripheral antinociceptive activity of gabapentin based on all groups. GBP, gabapentin; Cypro, cyproheptadine; L-Arg, L-arginine; Nalox, naloxone; L-NAME, L-nitro arginine methyl ester. *P<0.05 compared to control group. Values are mean ± SEM (n=7).

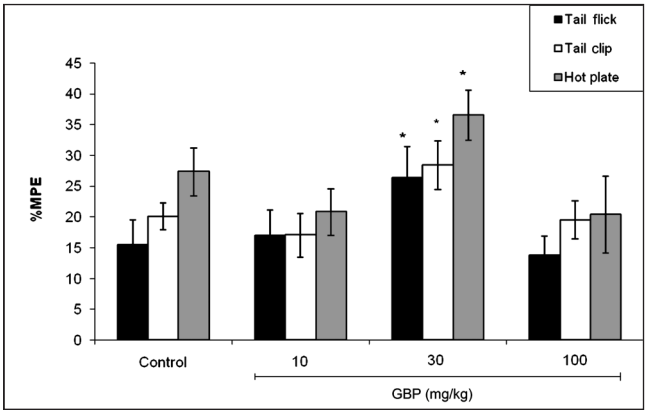

Fig. 3. The central antinociceptive activity of gabapentin at different doses. GBP, gabapentin; %MPE, % maximal potent effect. *P<0.05 compared to control; +P<0.05 compared to gabapentin (30 mg/kg). Values are mean ± SEM (n=7).

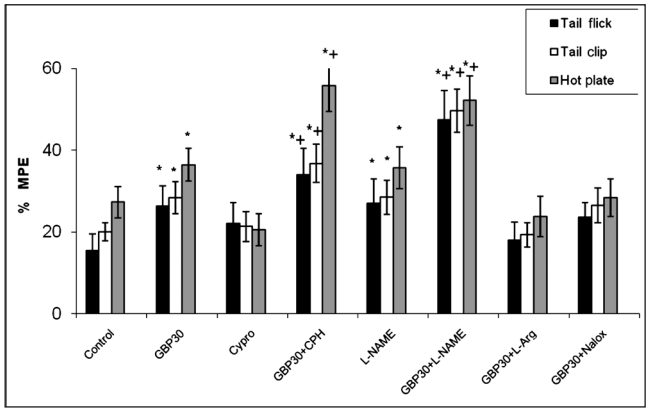

Fig. 4. The central antinociceptive activity in various groups. GBP, gabapentin; Cypro, cyproheptadine; L-Arg, L-arginine; Nalox, naloxone; L-NAME, L-nitro arginine methyl ester; %MPE, % maximal potent effect. **P*<0.05 compared to control; +*P*<0.05 compared to gabapentin (30 mg/kg).

with GBP (30 mg/kg), increased both the central and peripheral antinociceptive effects of GBP (Figs 2 and 4). Nitric oxide (NO) inhibition increased the central antinociceptive effect of gabapentin.

Studies in rat hippocampus slices: While gabapentin decreased PGE_2 at all doses significantly compared to the control groups (*P*<0.05), nNOS levels increased at all doses. Both decrease in PGE_2 and increase in nNOS were not dose-dependant.

L-NAME decreased PGE_2 significantly (*P*<0.05) at all concentrations and the combination of L-NAME and gabapentin decreased it further. Also, the gabapentin ($4x10^{-3}$ M) and L-NAME ($4x10^{-3}$ M) combination significantly decreased the PGE_2 level compared to all concentrations of L-NAME (*P*<0.05) (Table).

L-NAME increased the nNOS level significantly (*P*<0.05) at all concentrations compared to the control group but this effect was dose-independant. When used with gabapentin ($4x10^{-3}$ M), the nNOS level increased more compared to the control and gabapentin groups (*P*<0.05). Indomethacine reduced the PGE_2 level at all doses, but when used with gabapentin $4x10^{-3}$ M, there was no change. nNOS levels were increased by indomethacine at all concentrations and its combination with gabapentin compared to the gabapentin group and control group (*P*<0.05) (Table). Cyproheptadine caused an increase in PGE_2 at all doses, however, when used together with gabapentin ($4x10^{-3}$ M) it decreased PGE_2 compared to the control group (*P*<0.05) but not changed compared with the gabapentin group. nNOS levels increased with by cyproheptadine

Table. The levels of nNOS and PGE_2 in perfusates of rat hippocampal slices of all groups

Groups (M)	PGE_2 (pg/ml)	nNOS (µmol)
1-Control (SF)	136.07 ± 2.25	12.71 ± 0.42
2-Gabapentin $5x10^{-3}$	100.71 ± 1.27*	17.14 ± 0.50*
3-Gabapentin $4x10^{-3}$	106.85 ±1.74*	17.42 ± 1.21*
4-Gabapentin $3x10^{-3}$	108.38 ± 3.05*	18.71 ± 0.88*
5-L-NAME $5x10^{-3}$	107.35 ± 4.15*	22.28 ± 0.42*+
6-L-NAME $4x10^{-3}$	113.00 ± 2.30*	21.00 ± 0.52*
7-L-NAME $3x10^{-3}$	112.92 ± 4.33*	19.78 ± 0.51*
8-Gabapentin $4x10^{-3}$+L-NAME $4x10^{-3}$	99.35 ± 3.45*	24.07 ± 1.20*+
9-İndomethacine $5x10^{-3}$	90.57 ± 2.15*+	28.35 ± 0.95*+
10-İndomethacine $4x10^{-3}$	93.78 ± 2.84*	22.64 ± 1.02*+
11-İndomethacine $3x10^{-3}$	100.92 ± 2.73*	17.71 ± 0.74*
12-Gabapentine $4x10^{-3}$+ İndomethacine $4x10^{-3}$	93.57 ± 2.65*	27.71 ± 0.74*+
13-Cyproheptadine $5x10^{-3}$	154.00 ± 1.70*+	26.50 ± 0.58*+
14-Cyproheptadine $4x10^{-3}$	149.64 ± 3.43*+	26.57 ± 0.57*+
15-Cyproheptadine $3x10^{-3}$	146.78 ± 2.02*+	19.78 ± 0.48*
16-Gabapentin $4x10^{-3}$+ Cyproheptadine $4x10^{-3}$	104.28 ± 2.33*	21.42 ± 0.89*
17-Gabapentin $4x10^{-3}$+ Naloxone $4x10^{-3}$	129.28 ± 3.19	16.54 ± 2.93*

**P*<0.05 compared to control group; +*P*<0.05 compared to gabapentin ($4x10^{-3}$ M)

treatment at all concentrations (*P*<0.05), as also with cyproheptadine and gabapentin combination compared to the gabapentin group and control group (*P*<0.05). Naloxone has increased the PGE_2 level, which was reduced by gabapentin, to the control levels. However, no significant change was seen in the nNOS levels compared to the gabapentin groups (Table).

Discussion

Clinical and experimantal studies have revealed that gabapentin is effective in all kinds of neuropathic pain[3,4]. Although the spinal antinociceptive mechanism of gabapentin remains unclear, several hypotheses have been suggested. It has been reported that GBP decreases glutamate and glutamergic synaptic transmission presynapticaly[15]. Previous studies have shown that gabapentin increases the concentration, the rate of synthesis, and the release of GABA. However, intrathecal administration of $GABA_A$ and $GABA_B$ receptor antagonists did not reverse the anti-allodynic effects produced by gabapentin[16].

Gabapentin, in various doses, showed peripheral antinociceptive activity, and at central level in the 30 mg/kg dose as shown earlier[15]. Peripheral effects particularly at levels of α2δ-1 subunit in the dorsal root ganglia (DRG) and dorsal spinal cord are known to be increased in rat models of peripheral neuropathies[17]. While 30 mg/kg dose of gabapentin revealed a central antinociceptive effect, when used in combination with cyproheptadine and L- NAME the effect was more pronounced. With combination of gababentin and naloxone, no significant difference was observed in the spinal or supraspinal central antinociceptive effect compared to control. The responses observed in the hot plate test were believed to be supraspinally organized. The tail clip and the tail flick to radiant heat assays indicated that the spinal responses[18].

Gabapentin (30 mg/kg) combined with naloxone in our experimental study did not reveal any peripheral effect. However, Hansen *et al*[19] observed that gabapentin at spinal level prevents opiod tolerance, and reveals a better spinal analgesic effect compared to single use[6], and that the antinociceptive effect of gabapentin is reversed with naloxone[16]. These studies were conducted with neuropathic pain models.

In our study cyproheptadine at a dose of 2 µg/kg exhibited only peripheral antinociceptive effect. Gabapentin (30 mg/kg) showed a central antinociceptive effect at the supraspinal and spinal levels mediated through cyproheptadine. However, cyproheptadine did not show peripheral antinociceptive activity in combination with gabapentin. Gabapentin augments the level of serotonin[20], and intrathecal serotonin produces an antinociceptive effect being mediated through serotonin receptors[4,21].

L-arginine reduced the peripheral antinociceptive effect of 30 mg/kg gabapentin but did not change its central effect. L-NAME increased the central antinociceptive effect of 30 mg/kg gabapentin. However, it did not change its peripheral effect. NO inhibition strengthened the central antinociceptive effect of gabapentin.

Naloxone did not change the central and peripheral effects of gabapentin in the present study. Rettori *et al*[22] suggested the involvement of L-Arg- NO pathways in gabapentin antinociception.

In rat hippocampus slices gabapentin increased nNOS in all concentrations compared to the control group. Benzodiazepines have been shown to increase nNOS in the mouse elevated plus-maze[23]. Gabapentin has a GABAergic transmission like benzodiazepines. Both L-NAME and its combination with gabapentin increased nNOS levels. Taylor *et al*[24] in their study on human and rat brain NMR spectroscopy indicated that gabapentin increased GABA synthesis. There is evidence for interaction between GABA and NO, but the majority of such studies focus on NO modulation of release of GABA[25].

nNOS levels were increased by indomethacine at all concentrations and its combination with gabapentin compared to the gabapentin alone and control group. Indomethacine is NSAIDs and shows antiinflammatuar characteristics and is analgesic and antipyretic. In a study using the Western blot technique it was observed that nNOS expression in lipopolysaccharide (LPS) treated rat cerebellum was decreased compared to control groups of indomethacine[26].

Cyproheptadine in its various concentrations increased the nNOS levels compared to control group. Gabapentin combination masked the effect of cyproheptadine. While serotonin decreases nNOS in the central nervous system[20], cyproheptadine increases nNOS, as was also observed in our study.

The nNOS value measured with naloxone did not reveal a significant change compared to gabapentin groups. In an another study, interestingly, naloxone reversed the effect of gabapentin, which implicates that there are certain connections between the effects of gabapentin and opioid receptors[16].

Gabapentin at all doses decreased PGE_2 but not in a dose dependent manner compared to the control group. PGE_2 was decreased by L-NAME at all concentrations and by the combination of L-NAME and gabapentin. NO synthase leads to the production of NO, which subsequently activates cyclooxygenase and results in the production of PGE_2[22]. PGE_2 induced allodynia was also dose-dependently inhibited by L-NAME[27].

Indomethacine at all concentrations decreased PGE_2, but in combination with gabapentin ($4x10^{-3}$) did not change the result. It appears that PGE_2 release is not dependent on gabapentin[28]. Indomethacin blocked much of the lipopolysaccaride-induced prostoglandine increased in perfusate PGE_2 [29].

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