Polish Journal of Pharmacology
Pol. J. Pharmacol., 2003, 55, 803–806
ISSN 1230-6002

PRELIMINARY COMMUNICATION

EVALUATION OF INTERACTION BETWEEN GABAPENTIN AND BACLOFEN IN THE FORMALIN TEST IN MICE

Mirosław Czuczwar[1], Jacek Kiś[1], Jarogniew Łuszczki[2], Waldemar A. Turski[3,4], Krzysztof Przesmycki[1,#]

[1]Second Department of Anesthesiology and Intensive Therapy, Medical University, Staszica 16, PL 20-081 Lublin, Poland, [2]Department of Patophysiology, [3]Department of Pharmacology and Toxicology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, Poland, [4]Department of Toxicology, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-950 Lublin, Poland

Evaluation of interaction between gabapentin and baclofen in the formalin test in mice. M. CZUCZWAR, J. KIŚ, J. ŁUSZCZKI, W.A. TURSKI, K. PRZESMYCKI. Pol. J. Pharmacol., 2003, 55, 803–806.

Gabapentin and baclofen at doses not affecting motor performance, produced dose-dependent inhibition of both phases in the formalin test in mice. Isobolographic analysis revealed an additive interaction between the studied drugs in the second phase of the formalin test. Gabapentin given at doses effective in both phases of the formalin test significantly potentiated baclofen-induced motor impairment.

***Key words**: gabapentin, baclofen, nociception, formalin test, chimney test*

Gabapentin (GBP) is a novel antiepileptic drug, widely used in the treatment of neuropathic pain, with still poorly understood mechanism of action. It is known to act *via* increasing GABA (γ-amino butyric acid) synthesis, turnover, and non-vesicular release, but has no affinity for GABAergic receptors [9]. Also a specific calcium channel subunit (alpha 2 delta-1) was proposed as a target for GBP, which may act by decreasing neuronal calcium influx [12]. Spinal GBP treatment exerts dose-dependent antinociceptive effect in the formalin test (FT) [19]. Recently, GABAergic mechanism was proposed to explain the antinociception in the FT, which is an experimental model of facilitated pain processing responsible for allodynia and hyperalgesia in neuropathy [16]. Subcutaneous (*sc*) injection of dilute formalin into the mice hind-paw produces biphasic nociceptive response: phase 1 re-

correspondence; e-mail: anest2@panaceum.am.lublin.pl

flects an acute pain response and phase 2 represents the injury-induced spinal sensitization, responsible for facilitated pain processing [3]. On the other hand, spinal administration of the $GABA_A$ receptor antagonist bicuculline or the $GABA_B$ receptor antagonist phaclofen produces tactile allodynia and thermal hyperalgesia in normal rats [13]. The pathophysiological phenomena observed in some epilepsy and neuropathic pain models bear many similarities, therefore, the rationale for use of antiepileptic drugs in the symptomatic management of neuropathic pain disorders is justified, and it is not suprising that many antiepileptic drugs with GABAergic activity proved to be effective in models of neuropathy [10,19].

Baclofen (BAC), a derivative of GABA, is a potent $GABA_B$ receptor agonist, clinically used in the treatment of trigeminal neuralgia [7]. BAC is also known to possess dose-dependent protective activity in the FT [16]. Despite the antinociceptive activity of both GBP and BAC in the FT is well documented, yet to the best of our knowledge, their combination was never studied in any experimental model of neuropathic pain. Therefore, the antinociceptive effects of both drugs, given alone or in combination, were studied in the FT. Additionally, the effect of both drugs on motor performance was studied in the chimney test. Both drugs, GBP and BAC, possess GABAergic activity and we hypothesize that the interaction between them may have at least an additive nature.

All animal experiments, after acceptance by the Local Bioethics Committee, were performed on adult female Swiss mice weighing 20–24 g, in groups of 6–10 animals. The animals were kept under standard laboratory conditions with free access to food and tap water. All experiments were carried out between 09:00 a.m and 03:00 p.m. The mice were pretrained 24 h before behavioral tests and those unable to perform the chimney test were rejected from experimental groups. GBP (Parke-Davis Pharmaceutical Research, Plymouth, UK) was suspended in a 1% solution of Tween 80 and BAC (Baklofen, Polfa, Warszawa, Poland) was dissolved in 0.9% saline. Control animals received an equivalent volume of the vehicles. GBP and BAC were administered intraperitoneally (*ip*) 45 and 30 min respectively, before formalin and chimney tests.

The FT was performed according to Rosland et al. [17]; formalin, 20 µl of a 5% solution, was injected subcutaneously into the plantar surface of right hind paw using a 26-gauge needle. Immediately after formalin injection, animals were placed individually in Plexiglas chambers and observed for 30 min. The total time (s) spent licking the injected paw during periods of 0–5 min and 10–30 min after formalin injection was measured as an indicator of nociceptive behavior. For the dose-response analysis, data from phase 1 and phase 2 were analyzed separately. The effective dose producing a 50% reduction of nociceptive response in the control was defined as ID_{50}. The log dose-response curves were fitted using least square linear regression and the ID_{50} and 95% confidence limits (CL) for each drug were calculated [20].

The chimney test was used to assess the range of doses of the studied drugs producing motor impairment [1]. The animals had to climb backwards up a plastic tube (3 cm in inner diameter, 25 cm long). Motor-impairment was assessed as the inability of mice to climb backwards up the tube within 60 s. The values of TD_{50} (a toxic dose impairing motor coordination in 50% of mice) with respective 95% CL were calculated by probit analysis, according to Litchfield and Wilcoxon [11].

To determine the nature of the drug-drug interaction, we applied an isobolographic analysis [20]. GBP and BAC were administered in combination at fixed ratios of the ID_{50} dose for each drug (1:1). The experimental ID_{50} value and 95% CL for each drug and their combination were calculated. The theoretical additive ID_{50} doses, assuming simple additivity and 95% CL, were calculated according to Tallarida [20]. The value of the experimental ID_{50} for drug combination was compared with the respective theoretical additive ID_{50}. The ID_{50} values were considered to differ significantly ($p < 0.05$) from each other, if each ID_{50} value was outside the 95% CL of the other.

GBP and BAC administered alone *ip* produced a dose-dependent inhibition of both phases in the FT in mice. BAC inhibited nociceptive behavior in both phases of the FT at similar doses (Tab. 1). The doses of BAC (ID_{50}) effective in the FT were significantly, 3–4 times lower than in the chimney test (TD_{50}) (Tab. 2). Contrary to BAC, GBP inhibited nociceptive behavior in phase 2 at significantly lower doses (three-fold) than in the phase 1 (Tab. 1). The second phase of the FT is a composite of ongoing neuronal activity plus the generation of a facilitated state thought to result from sensitization of

the spinal cord (wind-up) and the GABAergic mechanism was proposed to explain the antinociception in this test [16]. Therefore, we decided to assess the interaction between BAC and GBP in the inhibition of the second phase in the FT. Previous drug-interaction analysis between BAC and antiepileptic drugs possessing GABAergic activity, using the FT, has demonstrated synergy [4] and additivity [5], so it is clear that the type of interaction can be determined with this test system.

Table 1. The inhibitory activity of gabapentin (GBP) and baclofen (BAC) alone or in combination in the phase 1 and 2 of the formalin test in mice

Treatment	ID_{50} (95% CL)[a]		Add./Exp.[b]
	phase 1	phase 2	
GBP	34.5 (18.5–64.2)	11.3 (6.3–20.3)	–
BAC	2.3 (1.5–3.5)	2.6 (1.7–4.0)	–
GBP + BAC	–	7.0 (4–10.5)	add.
GBP + BAC	–	4.7 (3.2–7.0)	exp.

[a] The ID_{50} value represents the total dose (mg/kg) resulting in 50% inhibition of the control response to formalin. [b] The theoretical additive (add.) or experimental (exp.) ID_{50} value of drug mixture in the formalin test (as described in the text)

Isobolographic analysis, using a fixed dose ratio, revealed not significant interaction between GBP and BAC in phase 2 of the formalin response. The experimental values of ID_{50} and 95% CL were not significantly different than the calculated additive doses for the second phase of the FT (Tab. 1), so we may conclude only an additive character.

Despite both BAC and GBP possess GABAergic activity, GBP increases GABA synthesis, turnover, and non-vesicular release, but has no affinity for GABAergic receptors [9]. The antihyperalgesic effects of BAC, but not GBP, are blocked by the selective $GABA_B$ receptor antagonist CGP56433A in models of chronic neuropathic (partial sciatic ligation) and inflammatory (Freund's complete adjuvant) pain in the rat [15]. Wind-up phenomena in the FT are mediated, at least partly, by neuronal calcium channels [14]. Previously Dunlap [6] has shown that the calcium channels are related to $GABA_B$ type receptors as their effectors, but not to $GABA_A$ type. Recently Luo et al. [12] suggested that the GBP's main site of action, responsible for inhibiting neuropathic pain response in the FT, is the neuronal calcium channel subunit (alpha 2 delta-1). Therefore, both drugs may exert the additive antinociceptive effect through the same receptor-effector complex.

BAC produced dose-dependent motor-impairment in the chimney test. GBP did not impair motor coordination even at the dose up to 1000 mg/kg (Tab. 2). GBP at the effective doses in the FT test significantly and dose-dependently potentiated motor-impairment produced by BAC (Tab. 2).

Table 2. Activity of gabapentin (GBP) and baclofen (BAC) given alone or in combination upon motor performance in the chimney test in mice

Treatment	TD_{50} (95% CL)	p[c]
GBP	1000 mg/kg[a]	–
BAC	12.7 (11.0–14.8)[b]	–
BAC + GBP 35	6.7 (5.7–8.0)	< 0.001
BAC + GBP 20	7.8 (5.6–10.9)	< 0.01
BAC + GBP 10	9.6 (8.2–11.3)	< 0.05
BAC + GBP 5	11.3 (10.0–12.8)	NS

[a] GBP maximal dose not affecting motor performance. [b] The TD_{50} value represents the toxic dose of BAC (mg/kg), impairing motor coordination in 50% of mice, given alone or in combination with the fixed dose of GBP (5, 10, 20 and 35 mg/kg). [c] Significantly or not significantly (NS) different from TD_{50} value of BAC given alone (Student's *t*-test)

The ineffectiveness of GBP given alone to produce motor-impairment in the chimney test is not surprising. Gąsior et al. [8] studied its influence on motor coordination in the inverted screen test and their results clearly showed that up to the dose of 1700 mg/kg, GBP did not produce motor-impairment, whilst its ID_{50} against cocaine-induced seizures was only 11.2 mg/kg. Since GBP did not produce motor-impairment in the chimney test, we were unable to evaluate its TD_{50} value and isobolographic analysis of interaction between both drugs in the chimney test could not be performed. Therefore, the effect of GBP upon BAC-induced motor toxicity was assed using different doses of BAC in combination with fixed doses of GBP (5, 10, 20 and 35 mg/kg). Our present results clearly have shown the significant synergistic potentiation of BAC-induced motor-impairment by GBP. GBP is a very safe drug in terms of producing neurotoxicity, both given alone or in combination with other

antiepileptics [2]. The antinociceptive effect of BAC in the formalin test and motor-impairment in the chimney test are probably due to a direct influence upon $GABA_B$ receptors in the spinal cord [18]. Therefore, we could not exclude the role of GBP-induced increase in GABAergic activity in potentiation of BAC-induced motor-impairment. On the other hand the pharmacokinetic type of interaction between GBP and BAC is not considered likely, because GBP did not elevate the free plasma levels of antiepileptic drugs, and GBP does not undergo a biotransformation and is entirely eliminated in urine [2].

The combination of GBP and BAC may have little clinical value. The observed synergistic effect of GBP and BAC on motor-impairment, may suggest that the side effects of BAC (e.g. sedation) will be significantly increased.

Acknowledgment. This study was supported by Lublin Medical University, grant No DS-283/02.

REFERENCES

1. Boissier J.R., Tardy J., Diverres J.C.: Une nouvelle méthode simple pour explorer l'action tranquillisante: le test de la cheminée. Med. Exp., 1960, 3, 81–84.
2. Borowicz K.K., Świąder M., Łuszczki J., Czuczwar S.J.: Effect of gabapentin on the anticonvulsant activity of antiepileptic drugs against electroconvulsions in mice: an isobolographic analysis. Epilepsia, 2002, 43, 956–963.
3. Coderre T.J., Vaccarino A.L., Melzack R.: Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. Brain Res., 1990, 535, 155–158.
4. Czuczwar M., Kiś J., Potasiński A., Turski W.A., Przesmycki K.: Isobolographic analysis of interaction between vigabatrin and baclofen in the formalin test in mice. Pol. J. Pharmacol., 2001, 53, 527–530.
5. Czuczwar M., Kiś J., Świąder M., Turski W.A., Przesmycki K.: Evaluation of interaction between valproate and baclofen in the formalin test in mice. Pol. J. Pharmacol., 2001, 53, 51–54.
6. Dunlap K.: Functional and pharmacological differences between two types of GABA receptor on embryonic chick sensory neurons. Neurosci. Lett., 1984, 47, 265–270.
7. Fromm G.H., Terrence C.P., Chattha A.S.: Baclofen in the treatment of trigeminal neuralgia: double blind study and long-term follow up. Ann. Neurol., 1984, 15, 240–244.
8. Gąsior M., Ungard J.T., Witkin J.M.: Preclinical evaluation of newly approved and potential antiepileptic drugs against cocaine-induced seizures. J. Pharmacol. Exp. Ther., 1999, 290, 1148–1156.
9. Jensen A.A., Mosbacher J., Elg S., Lingenhoehl K., Lohmann T., Johansen T.N., Abrahamsen B., Mattsson J.P., Lehmann A., Bettler B., Brauner-Osborne H.: The anticonvulsant gabapentin (neurontin) does not act through gamma-aminobutyric acid-B receptors. Mol. Pharmacol., 2002, 61, 1377–1384.
10. Laughlin T.M., Tram K.V., Wilcox G.L., Birnbaum AK.: Comparison of antiepileptic drugs tiagabine, lamotrigine, and gabapentin in mouse models of acute, prolonged, and chronic nociception. J. Pharmacol. Exp. Ther., 2002, 302, 1168–1175.
11. Litchfield J.T., Wilcoxon F.: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther., 1949, 96, 99–113.
12. Luo Z.D., Calcutt N.A., Higuera E.S., Valder C.R., Song Y.H., Svensson C.I., Myers RR.: Injury type-specific calcium channel alpha 2 delta-1 subunit up-regulation in rat neuropathic pain models correlates with antiallodynic effects of gabapentin. J. Pharmacol. Exp. Ther., 2002, 303, 1199–1205.
13. Malan T.P., Mata H.P., Porreca F.: Spinal GABA(A) and GABA(B) receptor pharmacology in a rat model of neuropathic pain. Anesthesiology, 2002, 96, 1161–1167.
14. Malmberg A.B., Yaksh T.L.: Effect of continuous intrathecal infusion of omega-conopeptides, N-type calcium channel blockers, on behavior and antinociception in the formalin and hot-plate tests in rats. Pain, 1995, 60, 83–90.
15. Patel S., Naeem S., Kesingland A., Froestl W., Capogna M., Urban L., Fox A.: The effects of GABA(B) agonists and gabapentin on mechanical hyperalgesia in models of neuropathic and inflammatory pain in the rat. Pain, 2001, 90, 217–226.
16. Rezayat M., Tabarrai E., Parvini S., Zarrindast M.-R., Pirali M.: Effects of CCK antagonists on GABA mechanism-induced antinociception in the formalin test. Eur. Neuropharmacol., 1999, 9, 9–14.
17. Rosland J.H., Tjølsen A., Maehle B., Hole K.: The formalin test in mice: effect of formalin concentration. Pain, 1990, 42, 235–242.
18. Shafizadeh M., Semnanian S., Zarrindast M.R., Hashemi B.: Involvement of GABAB receptors in the antinociception induced by baclofen in the formalin test. Gen. Pharmacol., 1997, 28, 611–615.
19. Shimoyama N., Shimoyama M., Davis A.M., Inturrisi C.E., Elliott K.J.: Spinal gabapentin is antinociceptive in the rat formalin test. Neurosci. Lett., 1997, 222, 65–67.
20. Tallarida R.J.: Statistical analysis of drug combinations for synergism. Pain, 1992, 49, 93–97.

Received: June 30, 2003; in revised form: August 14, 2003.