ACTA
NEUROBIOLOGIAE
EXPERIMENTALIS

Insulin impairs the anticonvulsive activity of carbamazepine against maximal electroshock-induced seizures in mice

Mohd Alaraj[1,3], Małgorzata Pieniak[2], Marek Kowalczyk[2]and Irena Kosińska[1]

[1]Department of Neurochemistry, Medical Research Centre, Polish Academy of Sciences, 5 Pawiński St., 02-106 Warsaw; [2]Department of Pharmacology, Military Institute of Hygiene and Epidemiology, 4 Kozielska St., 01-163 Warsaw; [3]Institute Research of Pharmacy, 8 Rydygier St., 01-793 Warsaw, Poland

Abstract. In view of the data indicating that insulin can modify penetration of some drugs across cell membranes and tissue barriers, particularly the blood-brain barrier, the aim of the present study was to evaluate the effect of insulin on both the anticonvulsant activity and the brain concentration of carbamazepine in mice suffering from seizures induced by maximal electroshock. The antiepileptic drug was administered per os in single doses either alone or in combination with insulin given as single intraperitoneal injections. To assess the anticonvulsant activity of carbamazepine the ED_{50} values were calculated. The results indicate that insulin given in doses up to 2 units/kg did not affect the convulsive threshold, whereas insulin applied at 2 units/kg led to a significant reduction in the anticonvulsant activity of carbamazepine, as judged by an increase in the ED_{50} value from 16.2 to 41.3 mg/kg. This effect was accompanied by the marked reduction in both the brain and blood concentrations of the drug. It is likely, therefore, that the inhibitory activity of insulin on the anticonvulsive function of carbamazepine is related not only to the effect of the former on the blood-brain transport of the latter, but also to insulin-induced modulation of the serum concentration of the drug.

Key words: blood- brain barrier, carbamazepine, insulin, mice, seizure

In mammals, the central nervous system (CNS) is separated from the circulating blood by the blood-brain barrier (B/BB). One of the crucial issues connected to the activity and therapeutic efficiency of some drugs targeted for the CNS is their limited permeability through the B/BB. Generally, brain concentrations of these drugs do not exceed a few percent of their serum levels. Thus, ways of elevating the passage of and the resulting concentration of drugs in the CNS is of utmost clinical importance (Rapoport et al. 1972, Salahuddin et al. 1988, Neuwelt 1989, Danysz 1992, Friden 1993). As shown by Danysz, Wiśniewski and co-workers, penetration of drugs across the B/BB can be enhanced by hormone peptides such as insulin (Danysz and Wiśniewski 1965, 1966, 1970, Wiśniewski 1968, Danysz et al. 1979). However, no data has been available so far as to the possible effects of insulin on the activity of antiepileptic drugs of which carbamazepine (CBZ) is one of the most frequently used (Spina et al. 1996), even though its effective anticonvulsant dose is close to the toxic level (Delgedo-Escueta et al. 1986, Calissi and Jaber 1995).

In view of the above, we aimed in the present study to assess the effect of insulin on both the anticonvulsant function and the brain and serum concentrations of carbamazepine in mice subjected to electroshock-induced seizures.

For the experiments, male outbred Ipf-Miz mice weighing 20-25 g were used. The animals were kept in colony cages under steady temperature and humidity and on a regular light-dark cycle. Mice were randomly assigned to the experimental or control groups consisting of 8-10 animals each. Carbamazepine (Polfa, Poland) was suspended in 0.5% solution of carbamethylcellulose and administered per os 30 min before the induction of electroconvulsions. Insulin (Insulinum semilente, Polfa, Poland) was diluted in 0.9% NaCl and injected intraperitoneally at the final dose of 0.5, 1.0, or 2.0 units/kg 1 h before the onset of electroshocks (as indicated by our unpublished results, this is the period necessary for insulin to exert its maximum effect on the activity of carbamazepine). As a rule, prior to the insulin injections 0.4, 0.6 or 1.0 ml of 40% glucose, respectively, was administered per os to prevent the possibility of hypoglycaemia-induced convulsions. Electroconvulsions were evoked according to the procedure described by Swinyard et al. (1952), using ear-clip electrodes and alternating current delivered by a Hugo Sachs stimulator type 221 (Freiburg, FRG). The stimulus duration was 0.2 s and the endpoint

was the tonic extension of the hind limbs. The convulsive threshold was defined as the CS_{50} value, i.e., the current strength in milliampers necessary to induce the hind limb extension in 50% of the tested animals. In mice receiving the anticonvulsant, the maximum electroshock (25 mA) was used and the ED_{50} (anticonvulsant dose effective in 50% of the animals) values were calculated. For determination of carbamazepine concentration, at the given time points the animals were sacrificed by decapitation, 1 ml blood samples were drawn and the brains were removed. Serum samples were obtained from the collected blood by centrifugation at 3,500 rpm for 5 min. The brains were homogenised at the ratio of 100 mg tissue: 100 μl H_2O (Witkiewicz 1995). The extraction procedure consisted of the addition of 0.75 μg of Secobarbital (internal standard), 200 μl of 1.5 M NaOH, and 50 μg of NaCl to 200 μl of plasma or brain homogenate, followed by 2 ml of ethyl acetate:chloroform (1:1) and vortex-mixing for 1 min. After centrifugation (1,800 g, 5 min), the organic phase was transferred to a conical tube and evaporated under a nitrogen flow at 50°C. The residue was reconstituted with 100 μl of n-hexane and 100 μl of the mobile phase (see below), vortex-mixed for 15 s, centrifuged (1,800 g, 10 min.), and the 100 μl aliquots of the serum or homogenised brain extracts were subjected to chromatography . The serum and brain levels of carbamazepine were determined using the Hewlett Packard Model 1050 liquid chromatography system with a Model HP 1050 Rheodyne injector 100 μl loop and a Hewlett Packard UV absorbance detector set at 220 nm. Chromatograms were obtained with use of a Model 3396A Integrator Hewlett Packard. All the analyses were performed on the 4.6 x 250 mm LC 18DB Supelco reversed-phase column with 5 μm-particles. The mobile phase was the acetonitrile /water solution (3:7 vol./vol.) at a flow rate of 1.6 ml/min (Witkiewicz 1995). The mean (± SD) concentrations of carbamazepine were obtained from at least 7 determinations. The differences in the brain and serum levels of the anticonvulsant were analysed statistically using the Students t test. The CS_{50} and ED_{50} values and the respective statistical significance of the differences were calculated using the probit analysis according to the method of Litchfield and Wilcoxon (1949).

As shown in Table I, insulin administered at 0.5 and 1.0 unit/kg led to the insignificant elevation of the ED_{50} values from 16.2 to 17.9 and 18.9 mg/kg, respectively, whereas the dose of insulin of 2 units/kg resulted in the significant increase of this value to 41.3 mg/kg.

TABLE I

Effect of insulin on ED_{50} of carbamazepine against maximal electroshock-induced seizures

Treatment	ED50 (mg/kg)	95% confidence interval
Carbamazepine	16.2	14.1-19.4
Carbamazepine + 0.5 u/kg insulin	17.9	14.5-21.9
Carbamazepine + 1.0 u/kg insulin	18.9	15.9-22.5
Carbamazepine + 2.0 u/kg insulin	41.3***	32.4-52.7

Carbamazepine was given p.o. 0.5 h before the maximal electroshock; ***$P<0.001$ vs. control (carabamazepine alone).

Insulin given at all the doses tested did not significantly affect the convulsive threshold, as indicated by the CS_{50} values of 5.50, 5.55, and 5.87 mA obtained respectively for 0.5, 1.0, and 2.0 units of insulin per kg, as compared to the control value of 5.72 mA (data not shown). In contrast, as shown in Table II, injection of insulin at the dose of 2.0 units/kg 30 min before the administration of carbamazepine resulted in a significant reduction of both the brain and serum concentrations of the anticonvulsant.

The obtained results indicate that insulin inhibits the anticonvulsive activity of carbamazepine in a dose-dependent manner. Since the post-insulin hypoglycaemia was always neutralised by the administration of glucose, the inhibitory effect of insulin seems to be independent of the serum glucose concentration. As previously reported by Danysz et al. (1966, 1979), insulin can both stimulate pharmacological activities and enhance the levels of a number of drugs (e.g., sodium salicylate and chlorpromazine) in the CNS. Thus, apparently our results with carbamazepine are not in agreement with the previous results. For this reason we decided to confirm whether insulin affects the electrical convulsive threshold or the pharmacokinetics of CBZ. It was found that the highest dose of insulin (in normoglycemia) appeared to be ineffective in modifying the convulsive threshold but resulted in the significant elevation of the effective anticonvulsive dose accompanied by the markedly pronounced reduction in the serum and brain concentrations

TABLE II

Effect of insulin on the serum and brain levels of carbamazepine in mice

Group	Concentration	
	(μg/ml)	(μg/g)
	Serum	Brain
Carbamazepine control	0.16 ± 0,025	0.58 ± 0.300
Carbamazepine + 2.0 u/kg insulin	0.09 ± 0.062*	0.18 ± 0.110**

*$P<0.05$; **$P<0.0$

of the anticonvulsant. Previous reports (Panatarotto et al. 1979, Monaco et. al. 1982) showed that plasma CBZ concentrations reach peak levels at 90 min postinjection, while brain concentrations reach this peak as early as 30 min postinjection. Moreover, both a prompt CBZ brain binding and sequestration in the CNS by the limited-exit kinetics were indicated (Monaco et al. 1982). For this reason we estimated the plasma and brain concentrations of CBZ exactly 30 min after injection. Obviously, our present results with carbamazepine do not support these observations but it may be argued that, in comparison to the drugs tested by Danysz et al. (1966, 1979), insulin affects the B/BB permeability of carbamazepine in a different way. Insulin may also enhance the levels of CBZ in other organ(s) than CNS, thereby inhibiting CBZ activity and its concentration in the brain. However, irrespectively of its mechanisms of action on CBZ activity, our results indicate that the insulin-induced modification of this anticonvulsants serum levels may play an important role in the brain distribution of at least several anticonvulsant drugs (own unpublished observations).

Other studies by Danysz et al. (1968) showed that if the application of insulin increased the effect of some drugs and their concentrations in the target organ, the activity and the concentration of these drugs would significantly decrease in diabetic (lack of insulin) animals (i.e., mice). Based on these data and on the results of the present investigation, it is possible to assume that the anticonvulsant activity, toxicity and the CNS level of carbamazepine may be significantly increased in non-insulin-treated diabetic patients. This assumption may be challenged by the results of the recent clinical study of

Nabavi et al. (1996), who demonstrated that in diabetic patients with neuropathy the effective dose of carbamazepine should be doubled as compared to non-diabetic patients. However these authors present no data on any pharmacological treatments received by the diabetic patients. Moreover, our results concern a model of acute insulin application, which is certainly not a case in diabetic patients. Further studies in diabetic animals are needed to resolve these controversies. Thus, regardless of the mechanism(s) responsible for the insulin-mediated impairment of the anticonvulsant function of carbamazepine, care must be taken during treatment of diabetic patients for epilepsy.

Calissi P.I., Jaber L.A. (1995) Peripheral diabetic neurophaty: current concepts in treatment. Ann. Pharmacother. 29: 769-777.

Danysz A. (1992) . The penetration of drugs to the Central Nervous System (in Polish). Problemy Terapii Monitorowanej 4: 119-125.

Danysz A., Wiśniewski K. (1965) Influence of insulin on the drug passage in to the tissue. Archs.Int. Pharmac.158: 30-38.

Danysz A., Wiśniewski K. (1966) The study of the insulin controlling and directing the chlorpromazine action and level in the brain tissue. Bioch. Pharmacol.15: 669-673.

Danysz A., Wiśniewski K. (1970) Control of drug action transport cell membranes. Materia Medica Polona 4: 1-10.

Danysz A., Wiśniewski K., Kiluk S. (1966) Influence of insulin on the anabolic action of Dianabol. Acta Physiol. Pol. 17: 841-847.

Danysz A., Wiśniewski K., Moniuszko J., Zarębski M., Buczko W. (1968) The action of some drugs in alloxanic diabetes (in Polish). Pol. Tyg. Lek. 23: 419-421.

Danysz A., Wutkiewicz M., Wierzba K. (1979) Influence of insulin on pharmacokinetics of mannosulfan in rats. Arch. Immunol. Ther. Exp. 27: 377-381.

Delgedo-Escueta A.V., Ward Jr A.A., Woodbury D.M., Porter M.J. (1986) New wave research in the epilepsies. Adv. Neurol. 44: 3-55.

Friden P.M. (1993) Receptor-mediated transport of peptides and the proteins across the blood-brain barrier. In: The blood brain barrier (Ed. W.M. Pardride). Raven Press, New York, p. 229-247.

Litchfield J.T.,Wilcoxon F.A. (1949) Simplified method of evaluating dose - effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113.

Monaco F., Mutani. R, Piredda S., Traccis S., Ramsay R.E. (1982) Brain uptake of carbamazepine in the cat. Epilepsia 23: 19-22.

Nabavi D.G., Georgiadis D., Stögbauer F., Michael M., Husstedt I.W. (1996) Meralgia paraesthetica. Dtsch. med. Wschr. 121: 834-838.

Neuwelt E.A. (1989) Implications of the blood-brain barrier and its manipulations. Basic science aspects (C.A. Neuwelt). Vol.1. Plenum Medical Book Company, New York, 403 p.

Pantarotto C., Crunelli V., Lanzoni J., Frigerio A., Quattrone A. (1979) Quantitative determination of carbamazepine and carbamazepine-10, 11-epoxide in rat brain areas by multiple ion detection mass fragmentography. Anal. Biochem. 93: 115-123.

Rapoport S.I, Hori M., Klatzo I. (1972) Testing of a hypotesis for osmotic opening of the blood-brain barrier. Am. J. Physiol. 223: 323-331.

Salahuddin T.S., Kalimo H., Johansson B.B, Olsson Y. (1988) Observations on exudation of fibronecitin, fibrinogen and albumin in the brain after carotid infusion of hyperosmolar solutions. An immunohistochemical study in the rat indicating long-lasting changes in the brain microenvironment and multifocal nerve cell injuries. Acta Neuropathol. (Berlin) 76: 1-10.

Spina E., Pisani F., Perucca E. (1996) Clinically significant pharmacokinetic drug interaction with carbamazepine. Clin. Pharmacokinet. 31: 198-214.

Swinyard E.A., Brown W.C., Goodman L.S. (1952) Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. Exp. Ther. 106: 319-330.

Wiśniewski K. (1968) Effect of insulin on the INH transport across the cell membranes in the perfused liver and isolated hemidiaphragm of rats. Biochem. Pharmacol. 17: 2117-2124.

Witkiewicz Z. (1995) Podstawy chromatografii. Wydawnictwo Naukowo Techniczne, Warszawa. p. 367.