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Novel long-term anticonvulsant treatment with gabapentin without causing memory impairment in mice

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ABSTRACT

We previously reported that administration of a single dose of gabapentin (GBP) immediately after training improves memory of mice in an inhibitory avoidance task (IA), whereas GBP administered repeatedly for 7 days impairs memory. This is in accordance with the observation that long-term clinical treatment with GBP may be associated with adverse cognitive side effects. In the present work we used a GBP-loaded poly(ε-caprolactone) implant, allowing controlled release of the drug and maintenance of constant plasma levels over 1 week. When GBP-loaded implants were inserted subcutaneously into mice, immediately after training in the IA task, memory consolidation was enhanced. Moreover, GBP released from implants had an anticonvulsant action against pentylenetetrazole-induced seizures. These results suggest that maintenance of stable GBP plasma levels could protect against seizures without causing memory impairment. Hence, the adverse cognitive effects might be avoided by stabilizing plasma levels of the drug.

1. Introduction

Long-term pharmacotherapy for the treatment of epilepsy may affect learning and memory processes [1]. A number of preclinical studies have attempted to characterize the cognitive effects associated with chronic exposure to newly developed or already commercialized drugs. Most of these investigations comprise the administration of a single or a limited number of doses (administered at different time points) and the later evaluation of the effect they have on certain behavior [2]. However, these studies are of limited relevance in the case of drugs involved in the treatment of chronic diseases [2,3].

Epilepsy is a very common and complex disorder characterized by anomalous neuronal discharges [4]. Cognitive disorders are a common complaint in patients with epilepsy, who constitute a high-risk population prone to develop different cognitive deficits. Treatment of epilepsy with antiepileptic drugs (AEDs) may exacerbate the occurrence of these deficits as a result of the proven reduction in neuronal irritability which may also be accompanied by impairment of neuronal excitability [5,6]. Hence, both the disease and its treatment might trigger cognitive impairment [7]. As the ultimate therapeutic goal is to control seizures with no or minimal side effects, the cognitive profile of an AED is an important factor in the selection of therapeutic options, and finding an effective treatment for epilepsy with a favorable cognitive profile remains a challenging issue. In this context, learning and memory impairments are commonly observed [5], and could be especially critical in the earlier stages of the learning process.

The anticonvulsant effects of AEDs can be measured by either (1) a decrease in seizure frequency, (2) an increase in the time elapsed until the presentation of convulsions, or (3) a decrease in the duration of the convulsive episode [8].

Gabapentin (1-aminomethyl cyclohexaneacetic acid) (GBP) is a drug structurally related to γ-aminobutyric acid (GABA), effective in the adjunctive treatment of partial epileptic seizures, with or without secondary generalization [9,10]. GBP is also widely used

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for the treatment of neuropathic pain and in off-label treatments [11], all of these being chronic regimens.

Since the appearance of GBP as an add-on therapy for epilepsy in 1993, many studies have aimed to determine its cognitive profile and the potential application of this drug to the management of other neurological disorders [10,11]. Several clinical and preclinical studies have provided evidence of the negative influence of GBP on memory performance after standard administration regimens [12–15]. Among preclinical studies, the potential contribution of GBP to memory deficit or enhancement has been a matter of comprehensive investigations by our research group [3,16–18]. A single administration of GBP enhanced memory consolidation of an inhibitory avoidance task in mice by disinhibition of central cholinergic pathways [16,17]. In addition, we demonstrated that the drug could produce anticonvulsant effects with no impact on memory retrieval [18]. In contrast, administration of GBP twice-a-day for a week, which results in a repetitive peak–trough profile, resulted in memory impairment [3]. This effect could be the result of impairment of memory retrieval processes caused by reduced activity of central cholinergic pathways. The reasons for the opposite effects of single- and multiple-dose treatments remain unclear.

In this work, we hypothesized that the repetitive peak–trough pharmacokinetic profile induced by twice-a-day injections could be the underlying cause of the memory impairment. Accordingly, a previously characterized drug delivery system (DDS) [19] was employed with the purpose of maintaining constant plasma levels of GBP over a week. We propose that constant low levels of GBP could be effective as add-on anticonvulsant treatment without causing memory impairment, and also improving retention performance by enhancing memory consolidation through disinhibition of the central cholinergic system.

2. Methods

2.1. Subjects

CF-1 male mice (FUCAL, Buenos Aires, Argentina) (age: 60–70 days; weight: 25–30 g) were individually identified and housed in stainless-steel cages, 10 per cage. Mice were maintained in a climatized animal room (21–23 °C) on a 12-h light/dark cycle (lights on 06:00 h), with ad libitum access to dry food and tap water. The experiments were conducted in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 80-23/96) and local regulations. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Behavioral procedures

Inhibitory avoidance behavior was studied in a one-trial learning, step-through type situation [20], which uses the natural preference of mice for a dark environment. This task is dependent on hippocampal function [21]. The apparatus consisted of a dark compartment (20 × 20 × 15 cm) with a stainless-steel grid floor and a small (5 × 5 cm) illuminated, elevated platform attached to its front center. Mice were not exposed to the apparatus before the learning trial. During training, each mouse was placed on the platform and received a footshock as it stepped into the dark compartment. The footshock training level was 0.8 mA, 50 Hz, 1 s. At the times indicated for each experimental group, the retention test was performed. Thus, each mouse was placed on the platform again, and the step-through latency (LTST) was recorded. The retention test was completed either when the mouse stepped into the dark compartment or when the mouse failed to cross within 300 s. In the latter case, the mouse was immediately removed from the platform and assigned a score of 300 s. The footshock was omitted in the retention test session.

2.3. Drugs

Poly(ε-caprolactone) (PCL, M_W 14 kDa; Sigma, St. Louis, MO, USA), gabapentin (GBP, Triquim SA, Argentina), scopolamine (SCOP, Sigma), pentylenetetrazole (PTZ, Knoll, Hungary), and solvents (analytical grade) were used as received. Mercaptoethanol and *o*-phthaldialdehyde were purchased from Sigma.

For the experiments involving injections, drugs were dissolved in saline solution immediately before use and administered intraperitoneally (10 mL/kg). Controls received the same volume of saline solution. The experiments were blind with respect to the drug treatments.

2.4. Acute PTZ-induced convulsions

A single intraperitoneal dose of 45 mg/kg PTZ causes tonic–clonic convulsions [20,22] (experiment 2). After the injection of PTZ, animals were observed for 30 min. The latency to onset was taken as the time elapsed until the first clonic body seizure. The duration of convulsions was measured between the onset and the end of this seizure episode.

2.5. Kindling procedure

PTZ-induced kindling was monitored following the repeated intraperitoneal administration of the subconvulsant dose of 30 mg/kg for 16 days (until all mice developed stage 5 seizures) (experiment 4). After injection of PTZ, animals were observed for 30 min and the intensity of seizures was classified according to Racine's scale [23]: stage 1, immobility, eyes closed, facial clonus; stage 2, head nodding, more severe facial clonus; stage 3, clonus of one forelimb; stage 4, rearing with bilateral forelimb clonus; stage 5, generalized tonic–clonic seizures.

The number of repeated administrations required to develop stage 5 seizures and the percentage of mice reaching this stage each day were noted. Latencies to onset and duration of the episodes were also recorded for each test.

2.6. Drug delivery system: implant preparation

An implantable GBP-loaded DDS was designed and manufactured as previously reported [19]. Briefly, disk-shaped PCL polymer implants loaded with 20% (w/w) drug and coated with pure PCL were prepared by a melt/molding compression procedure. The resulting DDS (dimensions: 11 mm × 4.3 mm, 450 mg) was characterized for in vitro and in vivo release rates: 2.2–2.5 mg/day in vitro, 4.5 mg/day in vivo [19]. When the DDS is inserted into a subcutaneous pocket in mice, immediate release of drug induces moderate plasma levels, below 10 μg/mL; later, the DDS maintains plasma levels in the 1–5 μg/mL range for 7 days [19]. GBP-free matrices were manufactured similarly and used as controls. During the experiments, mice were monitored for physical condition (weight and behavior). When indicated, usually at the end of the experiment, blood samples were obtained from the ophthalmic sinus using heparinized capillaries and GBP plasma levels were determined. The implants were recovered from mice and dried under mild conditions (48 h at 37 °C) to assess the implant weight loss, which correlates with the total amount of drug released [19] (data not shown).

2.7. Determination of gabapentin levels in blood samples and hippocampal homogenates

Blood samples (50–100 μL) were collected from the ophthalmic sinus [24] in 0.5-mL PCR tubes containing 5 μL of heparinized solution and gently mixed. Blood samples were centrifuged (10,000 rpm, 10 min, 4 °C). Supernatant plasma (30 μL) was carefully separated and deproteinized with acetonitrile (30 μL) and 10 μL of zinc sulfate solution (10%).

Mice were sacrificed by cervical dislocation, brains were rapidly removed, and both hippocampi were dissected over ice [25]. The tissues were weighed and homogenized in 10 vol of ice-cold distilled water using a hand-held Teflon glass homogenizer (10–12 strokes). Homogenates were centrifuged (10,000 rpm, 10 min, 4 °C), and the supernatant was separated and deproteinized with 15 vol of acetonitrile and 1.5 vol of zinc sulfate solution (10%).

After deproteinization, the samples were recentrifuged (4000 rpm, 5 min, 4 °C), and 50 μL of the supernatant was derivatized with *o*-phthaldialdehyde by the successive addition of citrate buffer (25 μL, 50 mM), sodium borate (30 μL, 500 mM), 2-mercaptoethanol (10 μL, 1 μL/mL), and *o*-phthaldialdehyde (30 μL, 1 mg/mL). Derivatization was performed 10 min prior to injection into the high-performance liquid chromatograph (see below).

Levels of gabapentin in blood samples and hippocampal homogenate were measured by liquid chromatography with fluorescence detection using a Phenomenex ODS column (5 μm, C18, 250 × 4.6 mm; LUNA) and a Model LC304 fluorescence detector (Linear Instruments). The excitation and emission wavelengths used were 230 and 420 nm, respectively. The mobile phase was a mixture of pure acetonitrile and 0.02 mM sodium phosphate buffer (50:50), pH 4. The mobile phase was filtered through 0.2-μm type HVLP Durapore membrane filters (Millipore) and the residual air was removed from them by bubbling through helium. The volume injected into the chromatography system was 20 μL. The flow rate used was 1.4 mL/min, and the retention time of gabapentin was approximately 8 min [26].

The chromatographic method was tested for linearity, precision, and reproducibility. The method was linear in the range 0.1–100 μg/mL and the coefficient of variation was less than 20% at the lowest concentration. The limit of quantification of gabapentin was 100 ng/mL for plasma samples and 500 ng/mL for hippocampal homogenates.

Brain homogenate concentration values expressed in micrograms per milligram (μg of gabapentin per mg of tissue) were converted into values expressed in micrograms per milliliter (μg of gabapentin per mL of tissue), assuming that brain specific gravity is 1.0 g/mL [3]. This conversion allows comparison between absolute values of plasma and hippocampal levels. It was previously reported that concentrations of GBP in brain cytosol may be about 4- to 10-fold higher than those in the brain extracellular space (i.e., about 8-fold higher than in plasma) [3,10].

2.8. Experimental groups

2.8.1. Experiment 1

To evaluate the impact that sustained levels of GBP have on memory, four groups of 10 mice each were trained in the inhibitory avoidance (IA) task. In two of these groups, GBP-loaded or drug-free implants were inserted as described above immediately after training. In the other two groups, the insertion was delayed 3 h after the training procedure. Eight days later, mice were tested for retention (Fig. 1A). Two additional groups of 10 mice each were also trained in the IA task but did not receive the footshock (unshocked groups), and implants were inserted immediately afterward.

At the end of the retention test, blood samples were collected from mice in the groups treated with GBP, and immediately afterward, hippocampi were dissected to determine GBP levels.

2.8.2. Experiment 2

To assess whether repeated injection of GBP (and the consequent repetitive peak–trough pharmacokinetic profile) is the cause of the memory impairment, four groups of 10 mice were used. All groups were trained in the IA task as described above. All mice received either a GBP-loaded or a drug-free implant immediately after training. In addition, all mice were subjected to a repeated dose schedule (two daily doses, 12 h apart, for 7 days) either with GBP (50 mg/kg IP) or saline solution (SS) [3]. The first injection was administered immediately after surgery, and the last injection was given 12 h before the retention test, performed on day 8 after training (Fig. 2A). Immediately after the retention test, mice were injected intraperitoneally with a convulsant dose of PTZ (45 mg/kg) to assess the anticonvulsant effect of implants and injections [18].

In addition, to determine plasma and hippocampal levels of GBP after concomitant administration of the drug via implant and injections, 5 mice were inserted with a GBP-loaded implant. Eight days after implantation, 12 h after the last injection of GBP, blood samples were taken and hippocampi were dissected.

2.8.3. Experiment 3

We previously reported the involvement of muscarinic cholinergic receptors in the action of GBP during memory consolidation [17]. To investigate the actual memory process modulated by GBP released from implants, six groups of 10 mice each were trained in the IA task. Immediately after training, GBP-loaded or drug-free implants were inserted and mice were injected intraperitoneally with saline solution, a dose of scopolamine (SCOP) that does not produce effects on its own (0.5 mg/kg) [3], or methyl scopolamine (mSCOP, 0.5 mg/kg). Two additional groups were included, in which the only difference was that the injection of SCOP (0.5 mg/kg) was delayed 3 h after insertion of the implant. The retention test was performed 8 days later (Fig. 3A).

2.8.4. Experiment 4

To monitor the development of PTZ-induced kindling during the administration of GBP, four groups of 10 mice each were used (Fig. 4). All mice received either a GBP-loaded or a drug-free implant. In addition, all mice were subjected to a repeated dose schedule (two daily doses, 12 h apart, for 7 days) of either GBP (50 mg/kg IP) or SS [3]. The first injection was administered immediately after implantation. As the implants maintain constant plasma levels for 7 days [19], each implant was replaced by a similar new one on days 8 and 15. The kindling protocol was begun on day 9 by administering one single daily intraperitoneal injection of a subconvulsant dose of PTZ (30 mg/kg) in the morning, 20 min after injection of SS or GBP, and was continued until all mice developed seizures (Fig. 4).

All animals were monitored for weight, adverse effects, and any abnormal behavior.

2.9. Data analysis

Behavioral data are expressed as median latencies to step-through during the retention test, and were analyzed with the Kruskal–Wallis nonparametric analysis of variance; differences between groups were estimated with individual Mann–Whitney *U* tests (two-tailed) [27].

Values of latency to and duration of convulsions are expressed as means ± SEM, and were statistically analyzed by one-way ANOVA followed by the Newman–Keuls test. Plasma and hippocampal

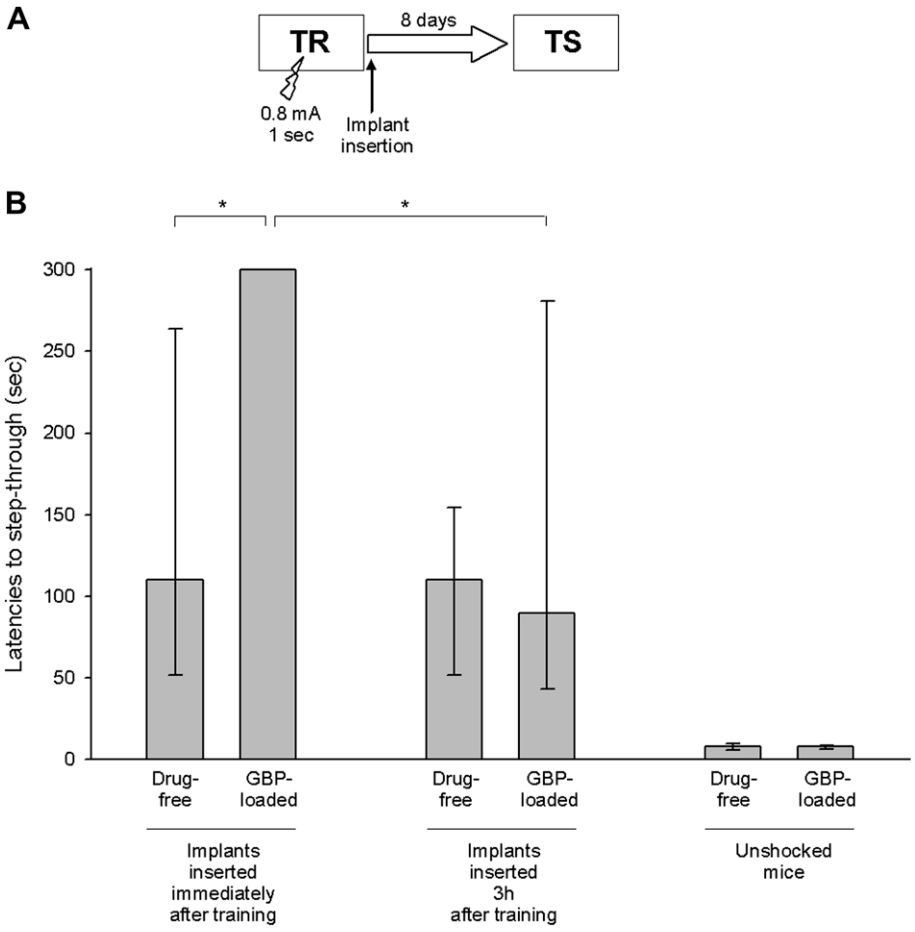

Fig. 1. Effects of GBP delivered from implants inserted immediately or 3 h after training in the inhibitory avoidance task. (A) Behavioral protocol. TR, training session; TS, test session. (B) Data are expressed as medians and interquartile ranges for $n = 10$ mice/group. $^{*}P < 0.05$.

levels of GBP were expressed as means ± SEM and were analyzed by one-way ANOVA.

In all cases, P values less than 0.05 were considered significant.

3. Results

3.1. Experiment 1

GBP released from implants improved retention performance in the IA task only when implants were inserted immediately after training ($P < 0.05$, when compared with drug-free implant group) (Fig. 1B). On the contrary, in the group receiving the implant 3 h after the training procedure, GBP did not enhance performance on the task ($P > 0.05$, compared with the drug-free control group). Moreover, mice treated with the GBP-loaded implant, though trained without receiving the footshock (unshocked groups), did not show increased retention latencies, indicating a lack of nonspecific effects of the drug. The improvement in performance obtained only when the drug-loaded implant was inserted immediately after training, but not when implantation was delayed, suggests that this enhancing effect is probably caused by enhancement of memory consolidation on the IA task and not by modification of memory retrieval.

Plasma levels of GBP were similar to those previously published in mice after insertion of implants [19], and hippocampal levels were in the expected range, that is, between three and four times higher than plasma levels, in accordance with previous results [3] (Table 1). No difference was found among the groups ($P > 0.05$).

3.2. Experiment 2

In animals treated with drug-free implants, the repeated administration of GBP (50 mg/kg, 12 h apart, for 7 days) produced an impairing effect on retention performance on the IA task ($P < 0.01$, compared with SS-injected mice) (Fig. 2B). In those mice that received the GBP-loaded implants and were injected repeatedly with SS, an enhancing effect was observed ($P < 0.01$, compared with the drug-free implanted group), in accordance with the results of experiment 1. However, mice that received GBP concurrently from implants and injections performed as well as controls on the task ($P > 0.05$), indicating that the drug-releasing implants could be preventing the deleterious effect of repeated injection of GBP on memory.

GBP had a significant anticonvulsant effect when administered via either implants or injections, as indicated by the increase in latency to the convulsive episode and the reduction of its duration ($P < 0.05$ or $P < 0.01$, compared with the control group). The maximal anticonvulsant effect was observed in mice that received the combined GBP treatment (implant plus injections) (Table 2).

Levels of GBP attained after the concomitant administration of the drug via implant and injections (measured 12 h after the last injection) were 2.45 ± 0.22 μg/mL for plasma and 8.24 ± 0.42 μg/

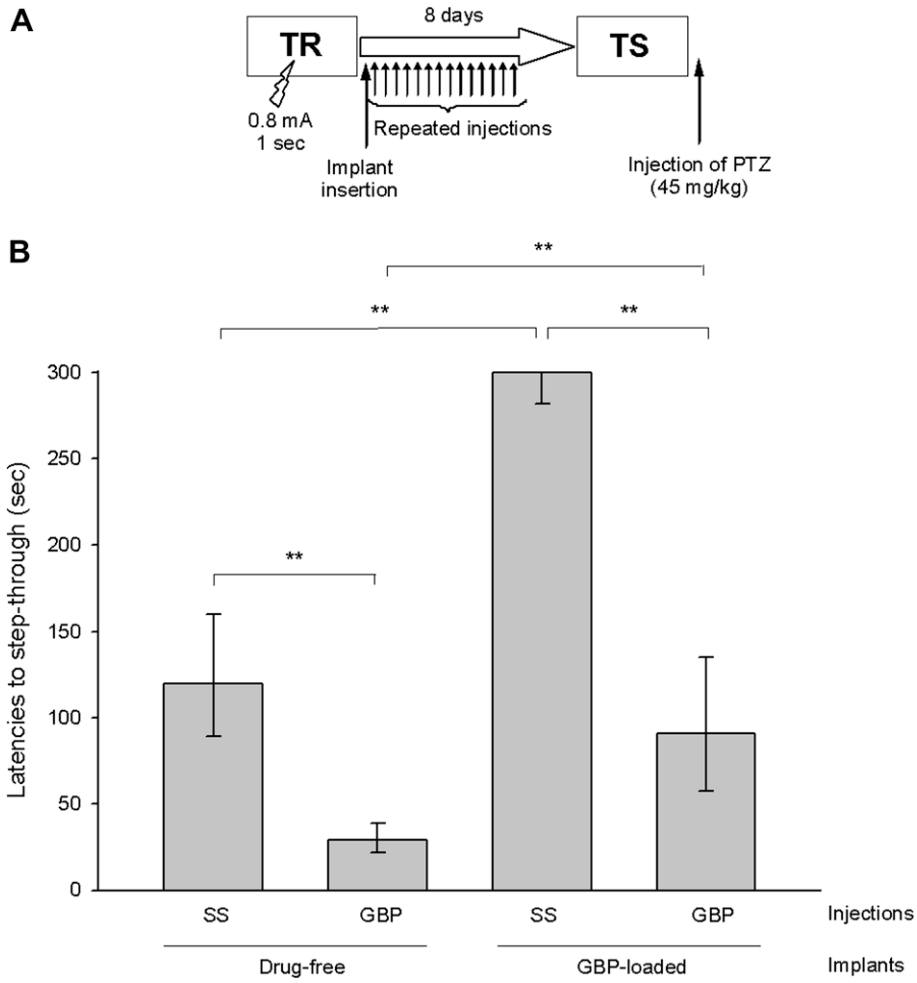

Fig. 2. Effects of GBP administered by implants and injections. (A) Behavioral protocol. TR, training session; TS, test session. (B) Data are expressed as medians and interquartile ranges for $n = 10$ mice/group. $^{**}P < 0.01$.

mL for hippocampus, similar to those obtained in mice that received the drug only from the implants.

3.3. Experiment 3

The enhancing effect on retention performance of the IA task produced by the GBP released from implants was successfully prevented by a dose of SCOP that by itself does not have impairing effects (0.5 mg/kg) (Fig. 3B). This effect was observed only when SCOP was administered immediately after implantation, and not when the injection was given with a 3-h delay. However, the same dose of mSCOP did not prevent the enhancing effect of GBP. These results indicate that muscarinic cholinergic receptors are involved in the action of GBP, and that the enhancing effect is probably exerted at the central nervous system level, through disinhibition of the cholinergic system during memory consolidation of this task.

3.4. Experiment 4

GBP delayed the development of seizures only when administered concurrently by implants and injections. In those mice that received GBP from implants alone and in those that received GBP only from injections, the only effect observed was reduction of the duration of the convulsive episodes [8] (compared with the drug-free implant plus SS injection group) (Table 3). Similar anticonvulsant effects were observed in these two groups ($P > 0.05$). Maximal effects were observed when mice received the drug from both implants and the injections (Table 3).

4. Discussion

Because of the opposite effects on memory performance of single and repeated administration of GBP [3], our research group has dedicated itself to finding a suitable explanation, although the neurobiological reasons underlying the difference between acute and chronic regimens remain elusive. In this sense, after a single intraperitoneal injection of 50 mg/kg GBP, short-lasting (1 h) high GBP plasma concentrations (up to 140 μg/mL 10 min after injection) followed by low levels (1–5 μg/mL, detectable from 1 to 4 h postinjection) were observed in mice. A single intraperitoneal dose of GBP results in memory enhancement, whereas a 1-week regimen of twice-a-day injections leads to memory impairment [3].

As GBP can potentially cause both enhancement and impairment of memory processes depending on the administration protocol [3], the question arises as to whether it is possible to develop a treatment schedule allowing long-term anticonvulsant therapy with GBP without causing memory impairment. With this aim in mind, a GBP delivery system was designed [19].

Poly(ε-caprolactone) is a hydrophobic and semicrystalline polymer that, because of its proven biocompatibility, low water affinity, and relatively long degradation time, is extensively used in diverse

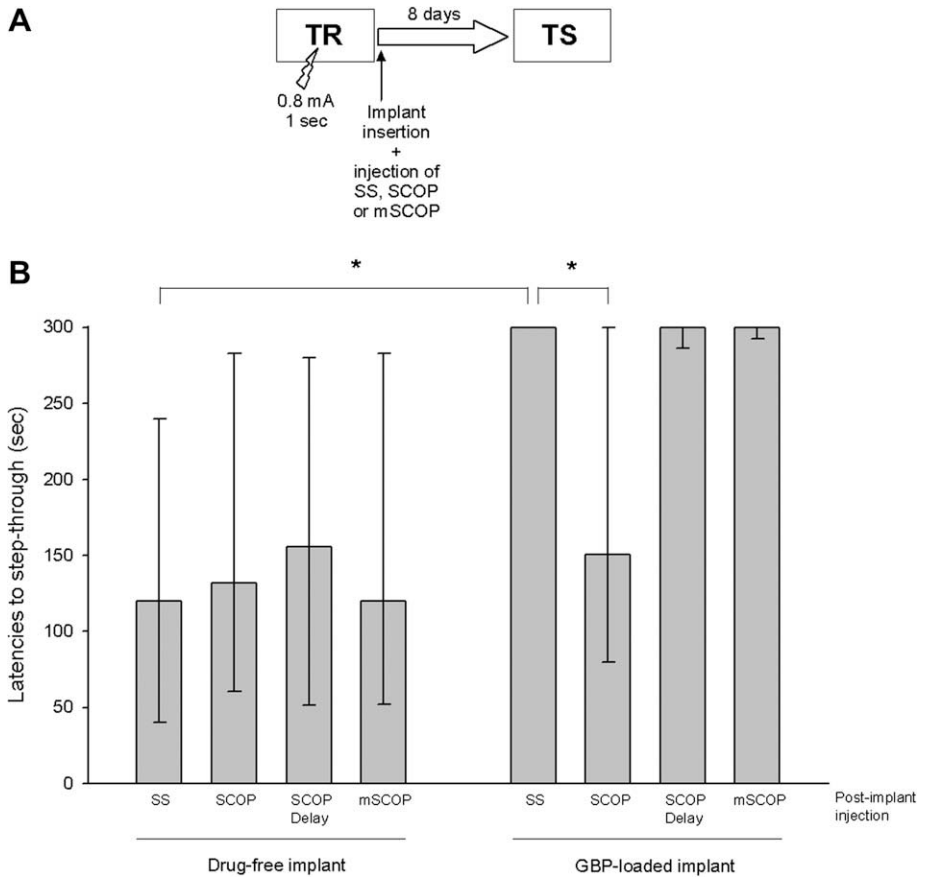

Fig. 3. Effects of an injection of SCOP (0.5 mg/kg) or mSCOP (0.5 mg/kg) either immediately or 3 h after implantation of the GBP delivery system (implants). (A) Behavioral procedure. TR, training session; TS, test session. (B) Data are expressed as medians and interquartile ranges for $n = 10$ mice/group. $^{*}P < 0.05$.

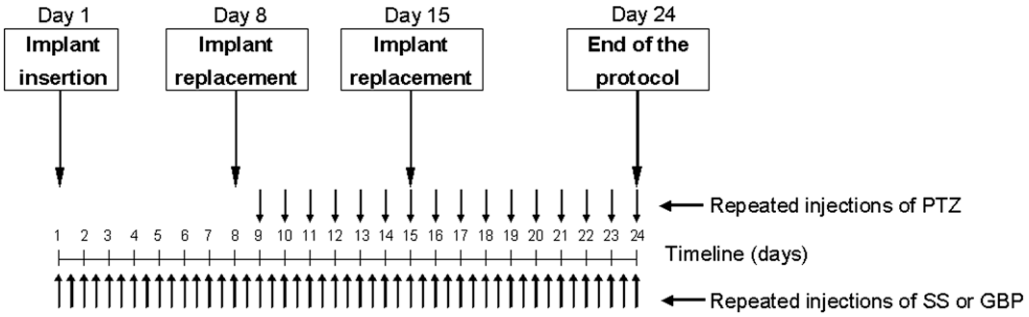

Fig. 4. Kindling procedure. Drug-free or GBP-loaded implants were inserted on day 1. Mice also received intraperitoneal injections of SS or GBP. Implants were replaced by similar ones on days 8 and 15. PTZ injections were begun on day 9. On day 24, 100% of the mice experienced seizures and the kindling protocol was ended.

Table 1
Plasma and hippocampal levels of GBP after treatment with drug-loaded implants.

	Plasma level (μg/mL)	Hippocampal level (μg/mL)
Implants inserted immediately after training (shocked mice)	2.18 ± 0.15	7.74 ± 0.53
Implants inserted 3 h after training (shocked mice)	2.09 ± 0.21	8.18 ± 0.32
Implants inserted immediately after training (unshocked mice)	2.33 ± 0.20	8.02 ± 0.42

biomedical applications [28]. PCL-based implants display high permeability to many drugs and remarkable biocompatibility, becoming a very versatile biomaterial in the design of DDSs [29–31]. Taking advantage of the unique properties of PCL, GBP-free and GBP-containing implants were produced and inserted subcutaneously into mice, ensuring sustained plasma levels of the drug for at least 1 week [19].

The main feature of our GBP delivery system was the attainment of GBP plasma levels comparable to those observed after the redistribution phase of intraperitoneal administration of 50 mg/kg (1–5 μg/mL) though without any exacerbated initial release. These implants allowed the administration of a total dose of GBP similar to that administered using the twice-a-day injection schedule [3] but maintaining stable GBP plasma concentrations for 7 days [19]. Thus, any effect observed on the memory of the mice receiving either implants or twice-a-day injections would stem from the different pharmacokinetic profiles and not from substantially different total doses.

Table 2
Anticonvulsant effect of GBP administered via implants and injections after a single administration of PTZ (45 mg/kg).

Implant	Injection	Percentage of convulsant mice[a]	Latency to first convulsive episode (s)	Duration of first convulsive episode (s)
Drug-free	SS	100	283.5 ± 22.9	10.3 ± 0.8
	GBP	100	341.0 ± 35.2[b]	8.0 ± 0.7[c]
GBP-loaded	SS	100	470.5 ± 41.9[b]	7.1 ± 0.5[b]
	GBP[d]	10	660[b]	3.0[b]

Note. PTZ was administered immediately after the retention test in the IA task, on day 8 after implantation. Data are expressed as means ± SEM, considering only those mice that had seizures.
[a]Percentages were taken over $n = 10$ mice/group.
[b]$P < 0.01$ and [c]$P < 0.05$ and in both cases when compared with the drug-free + SS group.
[d]In this group, only one mouse had convulsions. For this reason, SEM are not listed.

Table 3
Anticonvulsant effect of GBP administered using implants and injections after repeated administration of PTZ (30 mg/kg, one daily injection).

		Drug-free implant		GBP-loaded implant	
		SS injection	GBP injection	SS injection	GBP injection
Number of repeated administrations of PTZ required to develop seizures		4.6 ± 0.5[c]	6.4 ± 0.8[b]	6.3 ± 0.9[b]	10.3 ± 1.3[e]
Day 12	Percentage of convulsant mice	50	30	40	0
	Latency (s)	203.0 ± 11.9	228.3 ± 19.6	253.8 ± 59.5	—
	Duration (s)	10.9 ± 1.1	8.7 ± 1.2	8.3 ± 0.5	—
Day 16	Percentage of convulsant mice	100	80	70	40
	Latency (s)	174.0 ± 22.3[c]	207.5 ± 23.9[b]	211.4 ± 23.1[b]	422.5 ± 62.2[e]
	Duration (s)	11.6 ± 0.9[c]	8.0 ± 0.4[a,d]	8.4 ± 0.7[a,d]	4.9 ± 0.2[e]
Day 20	Percentage of convulsant mice	100	100	100	60
	Latency (s)	125.5 ± 10.5[c]	195.5 ± 33.8[b]	212.5 ± 30.2[b]	363.3 ± 46.2[e]
	Duration (s)	12.5 ± 0.9[c]	8.3 ± 0.6[a,e]	8.7 ± 0.4[a,e]	5.9 ± 0.7[e]
Day 24	Percentage of convulsant mice	100	100	100	100
	Latency (s)	102.5 ± 13.1[c]	156.5 ± 17.3[c]	161.5 ± 17.5[c]	283.5 ± 31.7[e]
	Duration (s)	12.9 ± 0.8[c]	9.0 ± 0.5[a,e]	8.9 ± 0.7[a,e]	6.6 ± 0.7[e]

Note. The times listed in the table are days after first implantation; administration of PTZ began on day 9 (see Fig. 4). Data are expressed as means ± SEM only for the animals that had seizures. Percentages are taken over 10 mice/group.
[a]$P < 0.05$, [b]$P < 0.01$, and [c]$P < 0.001$, in all cases when compared with the GBP-loaded implant + GBP injection group.
[d]$P < 0.01$ and [e]$P < 0.001$, in both cases when compared with the drug-free implant + SS injection group.

The enhanced retention performance on the IA task observed in mice that received GBP-loaded implants immediately after training, but not in mice that received the implants after a 3-h delay, suggests an effect of GBP on memory consolidation of this task. As further evidence supporting this suggestion, a dose of scopolamine ineffective on its own [3] prevented the improvement in performance only when given immediately after insertion of the implant, but not when delayed 3 h, also suggesting the involvement of central cholinergic pathways not only in the action of GBP, but also on memory consolidation of the IA task in mice [32].

In addition, nonspecific effects of the drug were ruled out because GBP-loaded implants did not increase retention latencies in the unshocked group. Taken together, these results are similar to those obtained after administration of a single injection of GBP [3,16,17]. Hence, following this treatment design, administration of GBP for a week could improve memory, that is, enhance memory consolidation, and not impair memory retrieval.

The results described above could also reveal the reason for the impairment of memory retrieval observed after repeated intraperitoneal administration. It is possible that successive peak–trough variations in plasma levels of the drug could produce repeated modifications of the degree of stimulation and/or inhibition of multiple neurotransmitter systems, including the cholinergic pathway. To avoid these variations in plasma concentrations of the drug, preclinical studies frequently employ subcutaneously implanted osmotic minipumps [33]. However, the devices commercially available do not allow administration of the high doses required in the present study. In the case of GBP, prodrugs [34,35] and gastroretentive tablets [36–38] have been found to be effective and safe for the treatment of pain associated with post-herpetic neuralgia. In animals, these approaches are less practical than parenteral devices, and therefore, we developed the new DDS [19].

As the total dose of GBP administered using either twice-a-day injections or implants is the same, the impairment of memory retrieval observed after the injections is probably due to the large variations in plasma concentrations of the drug repeatedly produced by each administration. This conclusion is supported by the fact that the impairment produced by repeated injections was prevented by the concurrent administration of GBP via implants.

In addition, GBP released from implants had an anticonvulsant effect on PTZ-induced seizures, when PTZ was either administered at a single convulsant dose or given repeatedly at a subconvulsant dose to induce kindling. When PTZ was administered at a convulsant dose, GBP, administered by injection or implant, increased latency to the onset and reduced the duration of seizures. The most efficacious anticonvulsant effect was observed when the drug was administered concurrently using both implant and injection.

When PTZ was used to induce kindling, animals that received GBP required more administrations than controls to develop stage 5 seizures. This effect was observed when the drug was administered concurrently via both implant and injection. Noteworthy, mice that received this concurrent treatment with GBP-loaded implants and intraperitoneal GBP injections performed as well as controls on the IA task. Treatment with GBP via implants alone or injections alone significantly decreased the duration of convulsive episodes. Together, these results indicate that implants could serve as an anticonvulsant treatment as effective as repeated injections [9], but not impairing memory.

In our experimental approach, the total dose of GBP administered by implants was similar to the dose administered by injections. The anticonvulsant actions observed after both methods of administration were similar, but the effects observed on retention performance were opposite. To explain these differences, the total amount of drug received by each mouse, the pharmacokinetic profile (peak–trough vs constant plasma levels), and the maximal plasma concentration attained after each administration of the drug (C_{max}) should be considered.

Mice that received GBP via implants or injections received the same amount of drug, but the C_{max} and pharmacokinetic profile differed. Those mice, however, experienced the same anticonvulsant effect. This may indicate that the anticonvulsant effect could be dependent on the total dose received by the experimental subject. In addition, GBP concurrently given by implants and injections had greater anticonvulsant effects. The fact that in this latter case GBP had a peak–trough pharmacokinetic profile (although with constant basal plasma levels) with slightly different C_{max} values, while the total dose administered was doubled, is also in accordance with the "total dose" interpretation.

On the contrary, memory performance seems not to be dependent on the amount of drug administered. Plasma levels (C_{max}) attained after single and repeated injections are the same, but the effects on memory are opposite [3]. This difference in memory effects suggests that the deleterious effect on memory may be produced by the large oscillations in plasma levels. The fact that mice that received GBP concurrently from implants and injections performed as well as controls on the memory task may be in accordance with this interpretation, as it may indicate that maintaining a basal plasma concentration of GBP could prevent the memory deficit, despite the oscillations in plasma levels caused by repeated injections.

Although these implants in their current form may not be useful in clinical practice, the development of suitable drug delivery systems for controlled release in humans could be useful not only in reducing the frequency of administration, but also in obtaining a better cognitive profile for the treatments, thus increasing the efficiency of the antiepileptic therapy, as we found for GBP.

In summary, our studies demonstrate that undesired central effects (memory impairment in this case) of some drugs might be a consequence of the pharmacokinetic profiles provided by the administration schedule, rather than by the drug itself or by the total administered dose. Hence, it may be possible to develop clinical strategies aimed at providing anticonvulsant effects without causing deleterious cognitive effects. Further studies should determine whether the proposed strategy is clinically relevant to avoiding the deleterious cognitive effects of chronic treatments.

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