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Dissociation of (−) Baclofen-Induced Effects on the Tail Withdrawal and Hindlimb Flexor Reflexes of Chronic Spinal Rats

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ADVOKAT, C., M. DUKE AND R. ZERINGUE. *Dissociation of (−)baclofen-induced effects on the tail withdrawal and hindlimb flexor reflexes of chronic spinal rats.* PHARMACOL BIOCHEM BEHAV **63**(4) 527–534, 1999.—We previously reported that the antinociceptive effect of the $GABA_B$ receptor agonist, (−)baclofen, in chronic spinal rats depended on the route of administration. That is, subcutaneous (SC) injections significantly increased the latency of the thermally elicited tail withdrawal (tail flick, TF) reflex, whereas spinal (intrathecal, IT) injections did not. The present studies attempted to determine the reason for this differential response. The possible contribution of a peripheral component to the systemic effect was evaluated, but was not supported by negative results of intradermal (−)baclofen injections (50 and 500 μg) into the tail skin of chronic spinal rats. A spinal site of action was indicated when pretreatment with 30 μg, IT of the $GABA_B$ receptor antagonist, phaclofen, significantly reduced the antinociceptive effect of SC (−)baclofen in both chronic spinal (5 mg/kg) and intact rats (2 mg/kg). Moreover, direct IT injections of (−)baclofen in chronic spinal rats produced a modest, but statistically significant increase in TF latency at doses of 0.06, 0.12, 0.3, and 0.6 μg, but not 1.2 μg. In the same spinal preparation, the flexor response was significantly reduced by IT injection of 0.6 and 1.2 μg, but not lower doses of 0.3 and 0.12 μg. These results provide the first quantitative, electrophysiological evidence of an antispastic effect of IT (−)baclofen in an in vivo, unanesthetized animal model. Second, the data show a separation between an antinociceptive effect of low spinal doses and an antispastic/muscle relaxant effect at higher doses, which may account for the results of our prior report. Finally, the data are also consistent with behavioral reports of antiallodynic/analgesic effects of low-dose baclofen, and may be relevant to the electrophysiological evidence of a preferential presynaptic action of low-dose (−)baclofen at the primary afferent synapse.

Tail flick Flexor Spasticity (−)Baclofen Phaclofen Spinal rat

THE $GABA_B$ receptor agonist (±) baclofen is presently the most efficacious, clinically available agent for the treatment of spasticity (18,19,21). Administration by either the systemic or spinal routes (2,8,21–24) provides a therapeutic benefit for spasticity arising from several etiologies, including spinal cord injury, multiple sclerosis, cerebral palsy, and cervical myelopathy. Yet, despite the fact that spasticity is the primary clinical indication for baclofen, relatively few experimental paradigms have been used to study its muscle relaxant properties in nonhuman animals (26,27,30). Most of the animal research on baclofen has examined the purported analgesic effect it exerts against a variety of noxious stimuli. These studies have reported that both systemic and spinal (i.e., intrathecal) administration produce analgesia in response to brief, transient nociceptive stimulation (3,4,11,12,35,38) as well as chronic inflammatory (9,28,29) or peripheral neuropathic pain [(29); for reviews, see (18,19,25)]. Most recently, baclofen has also been found to have an antiallodynic action after central (13,37) or peripheral injury (15).

Unfortunately, with the exception of some central pain syndromes (18,19,31) baclofen is not a very effective analgesic in humans. Yet, few in vivo animal models have been developed to investigate its more clinically relevant antispastic effect. For this reason, our laboratory recently incorporated a subjective rating scale of spasticity in our ongoing studies of antinociception in spinally transected rats (6). This was possible because rats develop a spastic condition within 3–4 weeks after spinal transection that is amenable to experimental analysis (1,20).

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The results of a previous study revealed an unexpected differential effect of the drug as a function of the route of administration (6). In chronic spinal rats both SC and IT administration qualitatively appeared to reduce spasticity. However, only the SC route produced an antinociceptive response of the thermally elicited tail flick reflex.

Those data led to the speculation that systemically administered (−)BAC produced antinociception, at least partly, by a peripheral mechanism (5). The present studies were initiated to test this hypothesis, by assessing the effect of (−)BAC after intradermal (ID) injection, under the skin of the tail. However, when the results did not support a peripheral site of action, additional experiments reexamined the effect of IT (−)BAC on the TF and the electrophysiological hindlimb flexor reflex in the chronic spinal preparation.

GENERAL METHOD

Subjects

Overall, a total of 145 (36 Intact; 109 Chronic) male albino Sprague–Dawley rats (Holtzman Laboratories, Madison, WI), weighing between 250–350 g at the start of the experiments, provided data for these studies. They were singly housed in plastic or suspended steel cages in a colony room maintained on a 12 L:12 D cycle, with dark onset at 1900 h. All animals had continuous access to food and water throughout the experiments. At the end of the experimental procedures, rats were euthanized by an anesthetic overdose or administration of CO_2. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University (Baton Rouge, LA).

Surgery

The animals were anesthetized using a mixture of isoflurane (AErrane, Anaquest, Madison, WI) and oxygen at a flow rate of 4 l/min. Anesthesia was maintained by allowing the animal to breathe a mixture of isoflurane and oxygen through a mask (flow rate 2 l/min). An incision was made so that the paraspinal muscles could be retracted, and a laminectomy performed between T6–T9. A 1–2-mm portion of the spinal cord was removed by excavation and replaced with Gelfoam to reduce bleeding, after which the incision was sutured and the skin closed with wound clips. After transection, spinal animals were placed in a room in which the ambient temperature was raised to approximately 80°F with a space heater, to maintain body temperature.

Chronic Spinal rats were tested at an average of 43.7 (±1.5) days after surgery. For the first 2–3 weeks, they were housed in the temperature-controlled room and their urine was expressed manually, twice daily, by the application of pressure to their bladders. All animals were also given 0.25 ml of the antibiotic Sulfatrim Pediatric Suspension orally (Barre-National, Baltimore, MD) once daily to prevent bladder infection. As reported previously (6), within approximately 2 weeks, transected animals regained bladder and temperature control, and the supportive postsurgical measures were no longer needed.

Drug Administration

All drugs (obtained from Research Biochemicals, Inc., Natick, MA) were freshly prepared and dissolved in 0.9% saline (SAL). For systemic administration, the desired dose was injected either SC in the lower back, or intramuscularly (IM) bilaterally, half of each dose into each extensor caudae medialis muscle at the base of the tail (7), in a volume of 1 ml/kg of body weight. For ID injections the desired dose was injected directly under the skin of the tail in a volume of 10 μl of saline, via a 30-gauge needle (17). Intrathecal injections were performed by anesthetizing the animals with isoflurane, and the required solutions were administered in 10 μl of SAL, directly onto the spinal cord, by insertion of a 26-gauge needle attached to a Hamilton microsyringe into the lumbar intrathecal space, as previously used with chronic spinal rats [(1), and references therein].

Behavioral Assessment: Tail Flick

For tail flick (TF) assessment, noxious stimulation was produced by a beam of high-intensity light focused on the tail (iitcc, Inc. Woodland Hills, CA). The response latency of tail withdrawal was measured automatically, and was defined as the interval between the onset of the thermal stimulus and the abrupt flick of the tail. A 14 s limit was imposed to prevent tissue damage, such that animals not responding within 14 s were removed from the apparatus and assigned a response latency of 14 s. Each score on the TF test consisted of the mean of at least three trials with the beam applied to different sites on the tail for each trial to further minimize tissue damage.

Electrophysiological Assessment: Flexor Reflex

Chronic Spinal rats were suspended in a torso sling apparatus (Harvard Apparatus, Natick, MA), such that all four limbs hung freely. Two leads for recording were inserted percutaneously into the biceps femoris/semitendinosus muscle of one hindlimb, one ground lead was inserted subcutaneously in the thigh, and a pair of fine subcutaneous pin electrodes for stimulating were placed in the skin between the toes.

A baseline measure was taken approximately 30 min after the leads were implanted to determine the stimulus threshold. The stimulus parameters were five square wave shocks, at 500 Hz, 0.2-ms duration. After threshold determination, stimulus intensity was set at 2.5 × threshold. Stimulation and recording procedures were performed with a Nicolet Viking IV D system (Nicolet Instrument Corporation, Madison, WI). Following another 15–30-min interval, five predrug responses were elicited. Each response was rectified and integrated, within a time window of 200 ms, providing an index of the area under the curve (AUC) in mV × ms.

Statistics: Tail Flick

For statistical analysis, difference scores were calculated by subtracting the predrug TF latency from the postdrug latency for each rat. Thus, data consisted of a difference score for each animal at each time point. These scores were also converted to Percent Maximal Possible Effect (%MPE) with the formula: postdrug–predrug latency/maximum latency (14 s) − predrug latency × 100. Time– or dose–effect relationships were plotted, summarizing the mean difference score (change in latency) or % MPE of each group. To analyze these data, separate one-way and/or two-way ANOVAs or paired *t*-tests were performed (or the nonparametric equivalents when indicated) to determine whether there was an effect of time, dose, or interaction. If the ANOVA results indicated a main effect of dose or time, post hoc (Newman–Keuls or Dunns) tests were performed to determine which groups differed (SigmaStat, Jandel, San Rafael, CA).

TABLE 1

MEAN CHANGE (±SEM) IN TAIL FLICK LATENCY (SECONDS) OF CHRONIC SPINAL RATS 15 MIN AFTER AN INTRADERMAL INJECTION OF EITHER 50 OR 500 μg (−)BACLOFEN, OR SALINE

	Injection		
	Rostral	< site >	Caudal
Saline ($n = 4$)	−0.80 (±0.3)	−0.50 (±0.7)	−0.60 (±0.7)
50 μg (−)Baclofen ($n = 4$)	−0.61 (±0.7)	−0.46 (±0.5)	0.34 (±0.5)
500 μg (−)Baclofen ($n = 5$)	+0.34 (±1.8)	+0.08 (±0.7)	+0.50 (±1.0)

There were no significant differences among the groups.

Statistics: Flexor Reflex

Baseline scores consisted of the mean AUC (mV × ms) of the five predrug responses for each rat. To analyze the drug effects, percent of baseline scores were calculated at 30 and 60 min after administration, with the formula: postdrug score/predrug score × 100, and a two-way repeated-measures ANOVA was performed on these values (Sigma-Stat, Jandel). For both the TF and flexor reflexes, results were considered statistically significant at $p \leq 0.05$.

EXPERIMENT 1

The objective of this study was to determine whether (−)BAC would produce an antinociceptive effect on the TF reflex after ID administration.

Subjects and Procedure

Predrug TF responses of Chronic Spinal rats were measured at three separate sites, at 1″ intervals along the tail, after which each animal was injected ID with either SAL ($n = 4$), 50 μg ($n = 4$), or 500 μg ($n = 5$) of (−)BAC, in the middle test site. TF latency was again measured at each of the three sites 15 min after drug administration. As shown in Table 1, there was no evidence of an antinociceptive reaction.

Although the results of this procedure indicated that neither dose of (−)BAC was antinociceptive, the possibility was considered that a transient effect might have dissipated within 15 min. Therefore, the procedure was repeated, with additional groups of Chronic Spinal animals, which were injected with the higher, 500 μg, dose of (−)BAC ($n = 7$) or SAL ($n = 6$) and tested immediately after the injection as well as 15 min later.

Results and Discussion

The results, summarized in Table 2, show a transient, albeit a significant, increase in TF latency immediately after the (−)BAC injection, relative to SAL controls, $F(1, 11) = 7.1$, for drug, which waned within 15 min, $F(1, 11) = 16.6$ for time; and 11.2, for the interaction. As indicated, post hoc tests confirmed that the increase was limited to the site of the (−)BAC injection. Although these data demonstrate a peripheral antinociceptive action of (−)BAC, it was concluded that the effect was most likely not physiologically relevant because it was restricted to the site of injection, the duration was so brief, and the dose required so high. This outcome prompted a reexamination of the antinociceptive effect of systemic (−)BAC.

EXPERIMENT 2

Subjects and Procedure

Separate groups of Intact and Chronic Spinal rats were pretested on the TF, then injected IM with either SAL ($n = 3$ and 4, respectively) or (−)BAC (5 mg/kg; $n = 3$ and 4, respectively), and retested 30 and 60 min later.

Results and Discussion

A two-way ANOVA indicated that there was a significant difference among the groups, $F(3, 10) = 6.2$, consisting of an increase in latency in both Intact and Chronic Spinal (−)BAC groups relative to SAL controls, which declined over time, $F(1, 10) = 5.1$, data not shown. These findings confirm that the systemic route of administration is effective for (−)baclofen-induced antinociception. Therefore, the next study was performed to evaluate the contribution of the spinal cord to the phenomenon.

EXPERIMENT 3A

The aim of this study was to determine if the antinociceptive effect of SC (−)BAC in Intact and Chronic Spinal rats

TABLE 2

MEAN CHANGE (±SEM) IN TAIL FLICK LATENCY (SECONDS) OF CHRONIC SPINAL RATS IMMEDIATELY AND 15 MIN AFTER AN INTRADERMAL INJECTION OF EITHER 500 μg (−)BACLOFEN, OR SALINE

	Saline Injection				Baclofen Injection		
	Rostral	< site >	Caudal		Rostral	< site >	Caudal
0 Min post injection ($n = 6$)	−0.18 (±0.8)	−0.68 (±0.7)	+0.10 (±0.7)	($n = 7$)	+1.89 (±0.8)	+3.10* (±0.7)	+2.21 (±0.6)
15 Min post injection ($n = 5$)	−0.28 (±0.2)	−0.80 (±0.2)	+0.08 (±0.8)	($n = 6$)	+0.80 (±1.3)	+0.58 (±1.0)	+1.22 (±0.6)

*There was a transient (0 min postinjection only) increase in latency at the site of the (−)baclofen injection, relative to the scores recorded 15 min after (−)baclofen, or 0 and 15 min after saline.

would be reduced by IT pretreatment with the $GABA_B$ receptor antagonist, phaclofen (PHAC). The dose of PHAC (30 μg) was chosen on the basis of a prior report (4) that demonstrated that IT injection of this drug produced a competitive and selective antagonism of BAC-induced antinociception in the TF test of Intact rats, which was similar in effect at 30 and 100 μg.

Subjects and Procedure

After a TF pretest, Intact rats were injected IT, under light anesthesia, with either 30 μg of PHAC (n = 5), or SAL (n = 5), prior to an SC injection of either 2 or 5 mg/kg (−)BAC. Separate groups of Chronic Spinal rats were also pretested on the TF. In this case, two groups received control IT SAL injections prior to either SC SAL (n = 5) or (−)BAC (5 mg/kg; n = 5), while two additional groups received the IT PHAC injection prior to either SC SAL (n = 4) or (−)BAC (n = 5). TF latency was then measured at 30, 60, and 90 min after the SC injection.

Results and Discussion

The results of spinal PHAC pretreatment on SC (−)BAC analgesia in Intact and Chronic Spinal rats is shown on the upper and lower half of Fig. 1, respectively. The 60-min time point was chosen for comparison in this and subsequent studies, first to ensure that there would be no residual effect of the anesthetic used for the IT injection (i.e., at 30 min) and second, to limit the possibility that the results, at least in Intact rats, were not confounded by rostral spread of the antagonist (i.e., at 90 min).

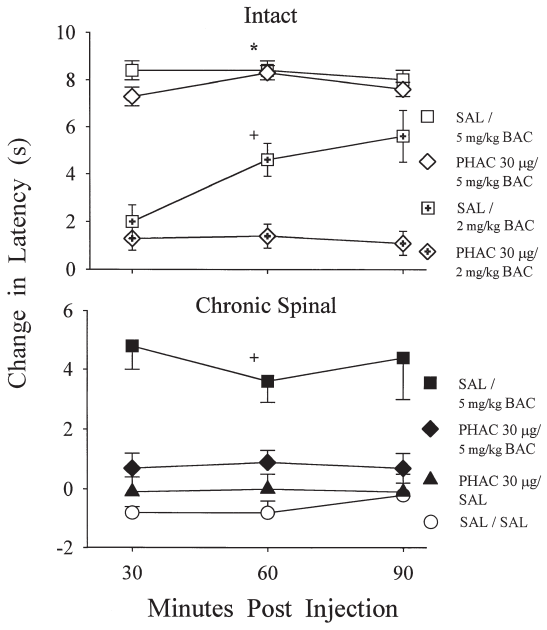

FIG. 1. Antagonism of systemic baclofen-induced [(−)BAC] antinociception by intrathecal administration of phaclofen (PHAC), in Intact and Chronic Spinal rats. Upper half: intact rats were injected SC with either 2 or 5 mg/kg (−)BAC, 15 min after intrathecal pretreatment with either saline (SAL) or 30 μg of PHAC. Lower half: Chronic Spinal rats were injected SC with either SAL or 5 mg/kg (−)BAC, 15 min after intrathecal pretreatment with either SAL or 30 μg of PHAC. Data are presented as the mean ± SEM of the difference in latency (post–predrug score) at 30, 60, and 90 min after the SC injection. n = 5 in each case, except PHAC–SAL, n = 4. Statistical analysis of the 60 min time point indicated a significant difference among the groups. *Significantly greater response than all other groups; +significantly different from all other groups, but not from each other.

Statistical analyses indicated a significant difference among the eight groups, $F(7, 38) = 48.4$. Post hoc comparisons showed that the response of both groups of Intact rats to 5 mg/kg (−)BAC (SAL and PHAC-pretreated, open squares and diamonds) was significantly greater than all other groups. Because the response of the Intact rats was larger than that of the Chronic Spinal rats (and was, in fact, above the cutoff ceiling of 14 s), it was not possible to determine the effect of phaclofen against the 5 mg/kg dose in Intact rats, or to compare this treatment between Intact and Chronic Spinal groups.

However, the effect of 2 mg/kg (−)BAC in Intact rats was not different from that of 5 mg/kg in Chronic Spinal rats, and the response of both of these groups was significantly different from that of every other group. This shows first, that the effect of 5 mg/kg SC (−)BAC on the TF is significantly reduced several weeks after spinal transection. A similar statistical decline to this dose, in Chronic Spinal, compared with Intact rats, was also seen in our previous report (6). Second, the data show that the effect of an SC dose of (−)BAC, which produces the same functional response in Intact and Chronic Spinal rats, will be antagonized, to the same extent, in both groups by the same (in this case, 30 μg) dose of IT phaclofen.

EXPERIMENT 3B

The results of the previous experiment indicated that the antinociceptive effect of SC (−)BAC in Chronic Spinal rats was primarily mediated by a spinal action of the drug. It was conceivable, however, that IT PHAC decreased TF latency in these animals because it produced a spastic reaction (elicited spontaneous tail flick reactions) rather than a pharmacological antagonism of antinociception. To test this, the effect of 30 μg IT PHAC (n = 5) on the flexor reflex of Chronic Spinal rats was compared to that of IT SAL (n = 5) at 30 and 60 min.

Results and Discussion

The results of this study (Fig. 2) show that although the flexor reflex is greater at 30 min after PHAC than after SAL, the overall difference between these two treatments is not statistically significant.

The data so far suggested a spinal rather than peripheral site of action for (−)BAC-induced antinociception after systemic administration, even in Chronic Spinal rats. Yet, in our previous report IT (−)BAC was not antinociceptive in these animals. However, in those studies, the lowest dose tested was 1.2 μg. Moreover, in those studies (−)BAC was administered IT through spinal catheters that had been implanted at the time of transection. It was possible that this additional trauma impaired the physiological condition of the rats and/or that the catheter impeded the flow of the injected drug in the spinal preparation.

EXPERIMENT 4A

Accordingly, the final experiments reevaluated the antinociceptive effect of IT (−)BAC in Chronic Spinal rats. In this case, several additional lower doses were included and spinal injections were made directly onto the cord. Moreover,

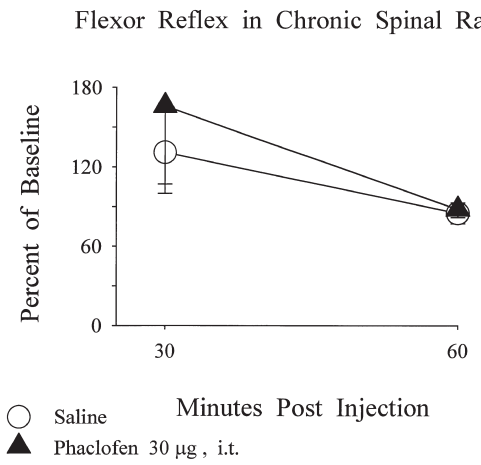

FIG. 2. Electrophysiologic recording of the flexor reflex in Chronic Spinal rats following IT injections of PHAC (30 μg, filled symbols, $n = 5$) or SAL (open symbols, $n = 5$). The data are presented as the mean ± SEM percent of predrug baseline for each group of rats at 30 and 60 min after injection. There was no difference between these conditions.

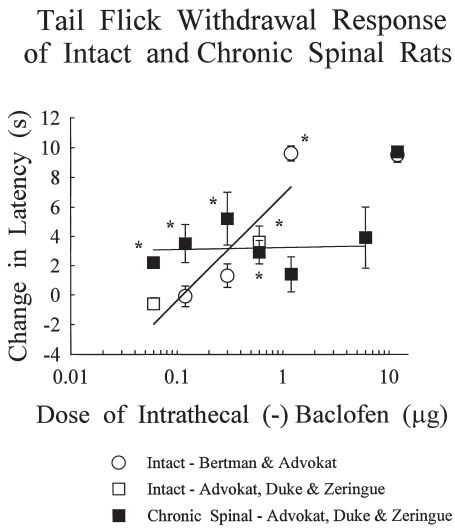

FIG. 3. Antinociceptive dose–response functions on the TF test for intrathecal (−)BAC in Intact (open symbols) and Chronic Spinal rats (filled symbols). The data represent the mean ± SEM of the latency difference (post–predrug score) at 60 min after injection. As indicated, this is a composite figure that includes previously reported data from Intact rats (6). Statistical analyses indicated that there was a significant dose-dependent effect of (−)BAC in Intact (0.06–1.2 μg) but not Chronic Spinal rats (0.06–6.0 μg). *Significant increase from predrug baseline.

in this study, many of the Chronic Spinal rats were also assessed on both the flexor and TF tests. This provided information about both the antispastic and antinociceptive effect of (−)BAC in the same rats.

Subjects and Procedure

The results from Chronic Spinal rats were combined with data from Intact rats, previously reported by Bertman and Advokat (6). However, because (−)BAC was administered via intrathecal catheters in the earlier article, rather than direct spinal injection, two additional groups of Intact rats (0.06 and 0.6 μg; $n = 5$ at each dose) were added to the present study to see if this procedural difference would affect the dose–response relationship of Intact rats.

Results and Discussion

A composite summary of the results can be seen in Fig. 3, which shows the change in TF latency 60 min after IT (−)BAC in separate groups of Intact and Chronic Spinal rats.

A one-way ANOVA was performed on the data from Intact groups (open symbols) for doses of 0.06 ($n = 5$), 0.12 ($n = 4$), 0.3 ($n = 6$), 0.6 ($n = 5$), and 1.2 μg ($n = 7$). Results indicated a significant effect of dose, $F(4, 26) = 39.6$, in that the response to 1.2 μg (−)BAC was greater than the response to all lower doses, and the effect of 0.6 μg was greater than that of the three lower doses. This dose-dependent antinociception was supported by two within-subject analyses, showing a significant increase above predrug baseline in response to 1.2 and 0.6 μg, $t(6) = 12.1$; $t(4) = 3.2$, respectively.

These results are consistent with numerous reports demonstrating an antinociceptive action of IT baclofen in Intact rats. The data also suggest that, at least in Intact rats, this phenomenon is not dependent on the presence of the intrathecal catheter, but is also produced by direct spinal injection.

Because some of the Chronic Spinal rats were tested on both the flexor and the TF (in that order) a two-way ANOVA was first applied to determine if the TF response was altered by prior performance of the additional test. There was no significant difference between the two conditions and the combined data are summarized in Fig. 3 (filled symbols).

A one-way ANOVA of the results from 0.06 to 6.0 μg indicated no significant difference among the groups on the TF test. In contrast to the Intact rats, Chronic Spinal rats did not show a dose-dependent antinociceptive response to IT (−)BAC.

However, on a within-subject basis, there was evidence of a modest, but statistical antinociceptive effect for most of the doses: 0.06 ($n = 5$; $t = 2.9$), 0.12 ($n = 10$; Wilcoxin $T = 42$), 0.3 ($n = 5$; $t = 2.9$), and 0.6 ($n = 10$; Wilcoxin $T = 55$), but not 1.2 μg ($n = 9$; $t = 1.2$). There were only three subjects at 6.0 and 4 at 12.0 μg, which were considered too few for statistical analysis.

EXPERIMENT 4B

The relationship between the TF and flexor responses is summarized in Fig. 4. For clarity of presentation, and to simplify comparisons with the flexor, the TF data (taken from Fig. 3) are shown as %MPE (filled symbols). This transformation did not change the outcome. Again, there was no dose-dependent antinociceptive effect of IT (−)BAC, but a significant increase from baseline at 0.12, 0.3, and 0.6, but not 1.2 μg.

At each of those doses, five of the rats received a concurrent flexor reflex test (open symbols). There was an overall significant difference among the four doses (Kruskal–Wallis $H = 10.2$), although there were no other individual differences. Within group comparisons showed that 0.12 and 0.3 μg did not affect the flexor, while this response was significantly reduced by 0.6 and 1.2 μg, to about 50% of baseline.

GENERAL DISCUSSION

The present studies were prompted by previous results from our laboratory, which seemed to show a differential ef-

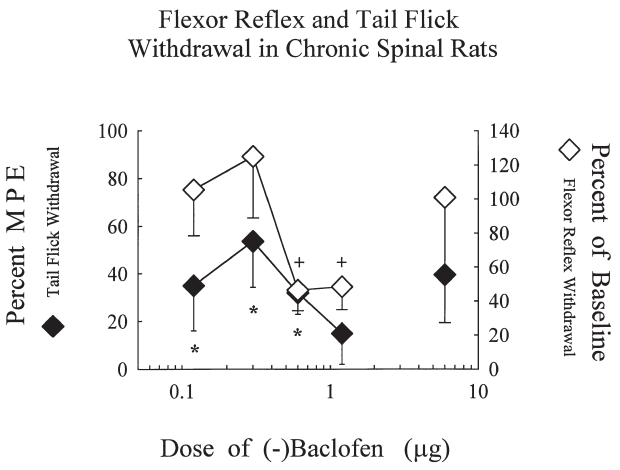

FIG. 4. Dose–response functions on the flexor reflex and tail flick withdrawal tests for intrathecal (−)BAC in Chronic Spinal rats. Results are presented as the mean % ± SEM of the predrug baseline and mean % MPE ± SEM, respectively, 60 min after injection. Tail-flick results are from the same animals whose latency data are summarized in Fig. 3. The flexor results were obtained from five of those animals at each of the indicated doses, prior to the tail flick tests. *Significant increase in tail flick response over baseline; +significant decrease of flexor response from baseline.

fect of SC and IT (−)BAC in Chronic Spinal rats (6). That is, only SC administration was antinociceptive on the thermally elicited tail-flick response.

One interpretation of those data was that after spinal transection there was a selective decline in the spinal antinociceptive potency of (−)BAC, and therefore, that the antinociceptive response of SC (−)BAC was mediated peripherally. The present experiments were initiated to evaluate that hypothesis, by assessing the effect of intradermal (−)BAC administration (injected under the tail skin) on the tail-flick reflex. However, as shown in Tables 1 and 2, the results of intradermal administration did not support a peripheral site of action for (−)BAC-induced antinociception after SC injection in Chronic Spinal rats.

This negative outcome, although not conclusive, led us to reexamine the phenomenon of (−)BAC-induced antinociception. The original observation was replicated, first, by IM and then by SC administration (Fig. 1). To evaluate the spinal contribution, Intact and Chronic Spinal rats were intrathecally pretreated with 30 μg of the $GABA_B$ receptor antagonist phaclofen (PHAC). When these groups were injected with equieffective doses of SC (−)BAC, the antinociceptive response was eliminated. The possibility that this antagonism was an artifact of a "prospastic" action of PHAC in the spinal rats was next ruled out, by comparing the effect of saline and PHAC on the flexor reflex of Chronic Spinal rats (Fig. 2). In support of previous results [in Intact, anesthetized, rats, (27,36)] there was no effect of this dose of PHAC on the flexor reflex.

The outcome of these studies consistently indicated a spinal site for SC (−)BAC-induced antinociception. This conclusion was then tested by administration of several IT doses of (−)BAC using the method of direct spinal injection, rather than the procedure of injection through a previously implanted catheter.

The results of this study (Fig. 3) confirmed that Intact and Chronic Spinal rats differed in their response to IT (−)BAC. In Intact rats this drug produced a dose-dependent analgesic reaction, which was evident at 0.6 μg and maximal at 1.2 μg. Although a 10-fold increase in dose to 12.0 μg also produced a maximal TF response in both Intact and Spinal rats, this was associated with such severe flaccidity that it was obviously an artifact of the profound muscle relaxation produced by this extremely large dose as originally noted by others (11,35).

In contrast to Intact rats, there was no dose-dependent antinociceptive response to IT (−)BAC in Chronic Spinal animals. However, on a within-subject basis, there was a differential effect of dose. Although 1.2 μg of IT (−)BAC did not produce an antinociceptive response in Chronic Spinal rats, there was a modest, but statistical increase above baseline after all lower doses. This result suggests that the TF latency increase after 1.2 μg in Intact rats may be due to a decrease in muscle tone, rather than a true analgesic response (4,12). As a result, the loss of this apparent antinociceptive action in Chronic Spinal rats may reflect the development of spasticity in these animals, which would counteract the decreased muscle tone and abolish the increase in latency.

This speculation is supported by the flexor results, which showed a significant decrease at that dose (Fig. 4). To the best of our knowledge, this is the first quantitative, electrophysiological demonstration of the antispastic effect of baclofen in an in vivo, unanesthetized animal model. Although the muscle-relaxant property of baclofen has been reported many times in a variety of experimental paradigms, those procedures used intact, usually anesthetized animals.

If that interpretation is correct, then the modest, albeit statistical, increase in latency produced by lower doses (≤0.6 μg) might represent a true antinociceptive response in both Intact and Chronic Spinal rats. This is also supported by the results of the flexor test, which showed no change at doses that produced a significant increase in TF latency (0.12 and 0.3 μg). Only the 0.6-μg dose produced a significant change in both measures, and an analgesic response in Intact rats, suggesting that the response in Intact animals may be due to a combined (sensory and motor) action of the drug. This would be consistent with previous reports in which spinal administration of (−)BAC was analgesic on the TF at doses of 0.1–1.0 mg (4,12,35).

Furthermore, these data may be particularly relevant to more recent investigations of (−)BAC in models of chronic or neuropathic pain (29) . Like the TF reflex, intrathecal doses of 0.3 and 1.0 μg (−)BAC were antihyperalgesic in the formalin test (9). Even lower doses of 0.1 and 0.3 μg were effective against the tactile allodynia produced by spinal root nerve ligation (15). The efficacy of low-dose (−)BAC against tactile allodynia was also demonstrated by Wiesenfeld-Hallin and colleagues in their model of ischemic spinal cord injury, photochemically induced by laser irradiation. Baclofen effectively reduced the "acute" phase of this allodynia, which developed during the first 24–48 h after spinal injury, at doses of only 0.01 and 0.1 mg/kg IP (13). [However, after cessation of the acute phase, a chronic phase of mechanical allodynic-like symptoms appeared, which was not relieved by 1 mg/kg (−)BAC; (37).] Moreover, even in normal rats, intrathecal administration of the $GABA_B$ receptor antagonist CGP 35348 produced a dose-dependent "pain-like" vocalization to innocuous tactile stimuli (14). Such evidence, implicating $GABA_B$ receptor function in chronic pain, may predict some clinical benefit, such as that reported for central pain produced by stroke or spinal lesion (31).

Recent electrophysiological evidence supports this hypothesis. It has been reported that low concentrations of baclofen depress synaptic transmission, primarily by a presynaptic action, because it occurs without any significant change in the passive membrane properties of the motoneuron (33,34). Furthermore, this effect of baclofen was blocked by the two $GABA_B$ receptor antagonists, CGP 35348 and CGP 55845A (33), but not by phaclofen (34). Higher concentrations elicited postsynaptic hyperpolarizations in at least some motoneurons (34). If these processes are relevant to (−)BAC-induced analgesia and muscle relaxation, respectively, then a selective effect of the antagonists against the antinociceptive TF response and the antispastic decrease in the flexor reflex would be predicted. This would support the existence of multiple $GABA_B$ receptor subtypes, and the possibility of developing a $GABA_B$ receptor agonist analgesic (10,19).

In summary, the data suggest that, at spinal doses below those that produce muscle relaxation (i.e., <1.2 μg) (−)BAC may exert a slight antihyperalgesic/allodynic action. Although larger doses may apparently produce antinociceptive reactions, such responses may be confounded in Intact rats with a decrease in muscle tone, especially when they depend on the ability to perform a normal motor response (e.g., tail flick, hot plate, limb withdrawal or flinch, writhing).

Finally, this interpretation may explain the apparent discrepancy between the effect of SC and IT (−)BAC on the TF reflex of Chronic Spinal rats. In brief, SC administration of 2–5 mg/kg may produce a spinal concentration of (−)BAC sufficient to reduce muscle tone in both Intact and Chronic Spinal rats and, that, in both populations, this is responsible for the increase in TF latency. This is consistent with an early study in intact, anesthetized rats, in which both a flexion and a nociceptive response were recorded after systemic baclofen administration. In that report, the analgesic ED_{50} was half that required for flexor reflex inhibition in the same animal (1.04 vs. 2.20 mg/kg, IP), supporting the conclusion that while low doses of systemic baclofen may exert a selective analgesic effect (16,32), there is a small separation between the analgesic and motoric actions of baclofen. Careful consideration of such differences will improve our understanding of these two important phenomena, particularly with respect to possible mediation by $GABA_B$ receptor subtypes and selective antagonists for pain and spasticity.

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