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Stereospecific actions of baclofen on sociosexual behavior, locomotor activity and motor execution

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Abstract. The behavioral effects of racemic baclofen and the R and S enantiomers were studied in order to determine whether the stereospecificity found in receptor binding studies also applies to the behavioral actions of the drug. Racemic and R-baclofen inhibited sexual behavior, locomotor activity and motor execution at relatively low doses while S-baclofen was completely inactive even when a dose 40 times higher than the minimum effective dose of R-baclofen was used. The R enantiomer seems to be twice as active as racemic baclofen. These data strongly suggest that the behavioral effects of baclofen are the result of an action at the GABA-B receptor. In order to differentiate the effects of baclofen on sexual interactions from those on nonspecific social interactions, the sociosexual behavior was observed with a castrated male or a receptive female as stimulus animal. R, S-baclofen had effects only upon sociosexual interaction with a receptive female. Moreover, the inhibitory effects of baclofen were restricted to behavioral items related to sexual interactions, primarily those constituting precopulatory behaviors. Since no effect was observed in social interactions with a castrated male, it is suggested that the inhibition of sociosexual behavior is not a consequence of impairment of motor execution. Rather it appears that baclofen has a specific inhibitory effect on behaviors associated with the initiation of copulatory activity. Therefore, once initiated, sexual behavior was not significantly modified by baclofen.

Key words: R(L)-Baclofen – S(D)-Baclofen – Sexual behavior – Locomotor activity – Motor execution

Previous studies in which the behavioral effects of GABAergic drugs were investigated have failed to establish any definite role for the GABA-A receptor. When GABA-A agonists are administered, a significant reduction in locomotor activity can be observed. A similar effect is found after administration of the GABA transaminase inhibitor γ-acetylenic GABA (GAG). However, neither the effects of GABA-A agonists nor those of GAG could be blocked by concurrent administration of bicuculline. Furthermore, since baclofen (a GABA-B agonist) has a strong inhibitory effect on locomotor activity, these results suggest that the reduction of locomotion is probably not due to stimulation of GABA-A receptors (Agmo and Giordano 1985). Similar data have been reported with regard to the effects of GABAergic drugs on sexual behavior, suggesting that the GABA-B rather than the GABA-A receptor is involved in the control of that behavior (Agmo and Paredes 1985).

In receptor binding studies, it has been shown that R-baclofen is the active stereoisomer. The R-enantiomer is as effective as GABA in displacing ^{3}H-baclofen from its binding site, while S-baclofen appears to be at least 100 times less active (Hill and Bowery 1981; Bowery et al. 1983). Furthermore, studies with cerebellar membranes have shown that S-baclofen does not significantly displace R-baclofen at any concentration used (Drew et al. 1984). Similarly, in electrophysiological studies of CA1 pyramidal cells of the rat, R-baclofen has been found to be at least 100 times more potent than S-baclofen (Haas et al. 1985). R-Baclofen is not only the active form in binding and electrophysiological studies; it also inhibited (as well as R, S-baclofen) the patellar, flexor and linguo-mandibular reflexes in cats. S-Baclofen is inactive in all these tests (Olpe et al. 1978 and references therein).

In the present experiment, the behavioral effects of racemic baclofen and the R and S enantiomers were studied in order to determine if the same stereospecificity described above concerning the biological activity and receptor binding of baclofen can be found with respect to baclofen-induced behavioral changes.

In the study mentioned above concerning the effects of GABAergic drugs on sexual behavior (Agmo and Paredes 1985), castrated animals were maintained at a submaximal level of sexual activity with weekly injections of a low dose of testosterone propionate. Thus very few animals showed the complete copulatory pattern. It was therefore considered to be of interest to observe the effects of baclofen on sexual behavior in animals with normal sexual activity. Furthermore, in a study concerning the effects of GABA transaminase inhibitors on sexual behavior it was found that whenever motor execution was impaired sexual behavior was also inhibited (Agmo et al. 1987). However, in the study by Agmo and Paredes (1985), baclofen inhibited sexual behavior at a dose that later was found not to affect motor execution. It is therefore possible that baclofen has a direct effect on sexual behavior. In order to explore this possibility it was decided to study the effects of baclofen on exploratory and sociosexual behavior of male rats exposed either to a receptive female or a castrated male, in addition to its effects on copulatory parameters. If the ef-

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fects of the drug were similar in both situations it could be supposed to act mainly via motor mechanisms, while if effects were obtained only when exposed to a receptive female, these effects could be specific for sexual behavior. This, together with the use of baclofen enantiomers, would allow us to obtain a more precise idea about the physiological function of the GABA-B receptor in the control of sexual behavior. Moreover, it was considered of interest to study the effects of baclofen enantiomers on locomotor activity and motor execution to further differentiate effects on sexual behavior from effects on motor performance.

Materials and methods

Subjects. Male Wistar rats from a local colony weighing 350–450 g, housed two to a cage, with free access to water and commercial rat pellets were used in all experiments. The animals were maintained under a reversed light/dark cycle (12/12 h) at constant room temperature (approximately 22° C). The animals to be used for studies of sexual behavior were observed with a receptive female for 30 min. Those animals that ejaculated during this period were castrated under ether anaesthesia and subcutaneously implanted with a 20 mm long testosterone (Sigma, St. Louis, MO) filled silastic capsule (0.062 i.d., 0.125 o.d.; Dow Corning Corporation, Midland, MI). Such an implant has been shown to maintain normal sexual activity for several weeks (Damassa et al. 1977). The animals were castrated in order to avoid possible endocrine effects of baclofen. It has been suggested that GABA plays a physiological role in the regulation of rat testicular androgen production (Ritta et al. 1987). Moreover, GABA has been found to inhibit the hypothalamic-pituitary-adrenal axis (Pericic et al. 1985) and it has been supposed that the release of several hypothalamic hormones might be modulated by GABA-ergic systems (DeFeudis 1984). Although castration does not eliminate the possibility of an endocrine effect of baclofen, it should be substantially reduced. Stimulus males used in tests of sociosexual interactions were castrated under ether anesthesia at least 2 months before being used.

Females used in tests for sexual behavior were ovarioectomized at least 2 weeks before use. They were injected with estradiol benzoate (Sigma), 25 μg/rat in 0.2 ml olive oil, 52–56 h before mating tests and progesterone (Aldrich, Milwaukee, WI) 1 mg/rat in 0.2 ml olive oil, 4–6 h before.

Males used in studies of locomotor activity or motor execution experiments were left intact. Extensive pilot studies have shown that there is no difference in drug effects on motor execution and locomotor activity between intact, castrated, and castrated, testosterone-implanted animals.

Drugs. R, S-Baclofen HCl and the enantiomers R-baclofen HCl and s-baclofen HCl (Ciba-Geigy, Basel, Switzerland) were dissolved in physiological saline and injected intraperitoneally (IP) in a volume of 1 ml/kg 20 min before the behavioral observation. Control animals were injected with saline. In studies of sexual behavior and sociosexual interactions the drugs were administered according to a Latin square design. That is, each animal in a given group received all the doses of a given drug (either the racemate or one of the enantiomers), in such a way that the effects of all doses plus saline were observed in a usually equal number of animals at each session. The interval between drug injections was 7 days, which should have been sufficient to avoid any effects being carried over from the previous treatment. The drugs used in motor execution and locomotor activity experiments were administered according to a counterbalanced design. No animal was treated with more than one enantiomer.

Procedure. In tests for sexual behavior the male was introduced into the observation cage (40 × 60 × 40 cm) where a receptive female had already been placed. The following parameters of sexual behavior were recorded: mount latency (time from introduction of the male until the first mount with pelvic thrusting); intromission latency (time from introduction of the male until the first mount with vaginal penetration); ejaculation latency (time from the first intromission until ejaculation); number of mounts with pelvic thrusting; number of intromissions (including the intromission associated with ejaculation). The tests lasted 15 min after introduction of the male or until ejaculation occurred. This test duration has been found to be adequate to evaluate drug effects on sexual behavior, including effects on mount, intromission and ejaculation latencies (Agmo and Paredes 1985; Agmo et al. 1987; Agmo and Fernandez 1989).

In tests for sociosexual interactions the male was introduced in the observation cage where a receptive female (sociosexual interaction) or a castrated male (social interaction) had already been placed. Besides recording the usual parameters related to sexual behavior, the frequency and duration of eight different behaviors were registered. The observed behaviors are based on the classification by Meyerson and Hoglund (1981) and include: rearing (standing on the hindlegs); sniffing (rapid movements of the head or whiskers while the animal explores); selfgrooming (licking or gently biting different areas of the fur, of the limbs, or of the genital area); grooming partner (licking or gently biting different areas of the partner's fur or limbs); genital exploration (sniffing or licking the partner's ano-genital region); pursuit (the experimental animal follows the stimulus animal keeping close contact, usually followed by a sociosexual interaction); resting (lying or standing still, without any particular overt behavior); incomplete mount (mount without pelvic thrusting). These behaviors were recorded using a keyboard with eight buttons. Each button was connected to an electronic equipment registering frequency and duration of each behavior. This equipment was located in an adjacent room. These tests lasted 10 min. The sociosexual interactions and sexual behavior were observed in different groups of animals in order to compare the sociosexual interactions realized with a castrated male to those realized with a receptive female. This would allow the separation of behavioral elements mainly related to social interaction from those mainly related to sexual interaction.

Locomotor activity was quantified in a circular arena (diameter 60 cm) surrounded by a 37.5 cm high wall. Six photocells covered with infrared filters were placed 2.5 cm above the grid floor at regular intervals. The locomotor activity of the rat was quantified as the number of beam interruptions in 10 min. Before drug treatments, the rats were habituated to the activity cage during three 10-min sessions, separated by at least 48 h. To activate the counters, a beam interruption of at least 200 ms was required. This means that rapid movements such as grooming or tail flicks were not registered. The activity count thus mainly reflects ambulatory activity.

In tests for motor execution, the animals were placed on top of a cylinder (diameter 16 cm) that rotated at 11 rpm. Whenever an animal fell down it was replaced on the cylinder about 5 s later. The motor execution was quantified as the number of falls in 3 min. Before drug treatments, the rats were trained in the apparatus for 15 min. During the first 5 min the apparatus rotated at 5 rpm, during the second 5 min the speed was increased to 8 rpm, and in the last 5 min the apparatus rotated at 11 rpm. The animals that fell down more than 5 times during the last 5 min were eliminated from the experiment. All tests were performed during hours 4–8 of the dark phase of the light/dark cycle under dim white light.

Statistical analysis. In test of sexual behavior the percentage of mounts, intromissions and ejaculation was analysed by Cochran's Q test and/or McNemar's test for the significance of changes or the binomial test when appropriate. The number of mounts and intromissions was evaluated with Friedman's ANOVA and/or the Wilcoxon matched-pairs signed-ranks test. In these analyses, all animals were included. The latencies were analysed by Kruskal-Wallis one-way ANOVA and/or the Mann-Whitney U-test. Here, only subjects from which data were actually obtained were included. Nonparametric tests were used for these parameters, since the distribution of the data strongly deviated from the normal distribution, and since error variances were not homogenous. In the case of the latencies, data were obtained from very few animals under some treatments, making the use of parametric tests inadequate.

The sociosexual behaviors were analysed by a two-factor MANOVA for repeated measures in one factor (doses of a given drug). The between-groups factor was stimulus animal (receptive female versus castrated male). In case of significant omnibus test, univariate ANOVA was performed for each variable. The Bonferroni correction was then applied in order to determine significant differences. In case of significant interactions in the omnibus test, multivariate tests for simple main effects were performed, followed by univariate ANOVAs with Bonferroni corrections for significance levels. When the factor dose had more than two levels, pairwise multivariate contrasts were performed using Hotellings T^2. When a significant result was obtained, pairwise univariate comparisons were made with the Tukey LSD procedure. The data for motor execution were evaluated with the Wilcoxon matched-pairs signed-ranks test. On vehicle tests, most subjects obtained a value of 0, producing a non-normal distribution of the data, and an error variance which was very small compared to that obtained after drug treatments. The locomotor activity was analysed by a t-test for repeated measures. When applicable, probabilities given are two-tailed.

Results

Sexual behavior

Racemic baclofen inhibited most aspects of sexual behavior at a dose of 5 mg/kg. The proportion of animals showing mounts and ejaculations was reduced (Table 1). Similarly, the number of mounts and intromissions was reduced. The increases in mount, intromission and ejaculation latencies did not reach statistical significance (data not shown). At 2.5 mg/kg, racemic baclofen had no significant effects. A dose of 1.25 mg/kg R-baclofen had no effect, whereas 2.5 mg/kg produced almost complete inhibition of sexual behavior (Table 2). The proportion of animals showing mounts, intromissions and ejaculation was much reduced, as well as the number of mounts and intromissions. The apparent increase in mount and intromission latencies did not reach statistical significance. The ejaculation latency was not analysed, since no animal achieved ejaculation (data not shown). When a dose of 10 mg/kg S-baclofen was administered no effects on sexual behavior could be detected (Table 3).

Table 1. Sexual behavior in male rats treated with R, S-baclofen 20 min before observation. $N=16$ at each dose

Behavior parameter	Vehicle	R, S-Baclofen 2.5 mg/kg	R, S-Baclofen 5 mg/kg
Mount percentage	93	68	62**[b]
Intromission percentage	75	56	37
Ejaculation percentage	43	37	6**[b]
Number of mounts[a]	3.8±0.84	2.6±0.79	0.8±0.37**[c]
Number of intromissions[a]	6.4±1.22	4.1±1.02	2.0±0.73**[c]

** Different from vehicle ($P<0.01$)

[a] Mean±SE
[b] McNemar's test
[c] Wilcoxon matched pairs signed-ranks test

Table 2. Sexual behavior in male rats treated with R-baclofen 20 min before observation. $N=18$ at each dose

Behavior parameter	Vehicle	R-Baclofen 1.25 mg/kg	R-Baclofen 2.5 mg/kg
Mount percentage	88	72	27**[b]
Intromission percentage	83	72	22**[b]
Ejaculation percentage	27	22	0*[b]
Number of mounts[a]	3.1±0.64	2.5±0.91	0.6±0.25*[c]
Number of intromissions[a]	5.3±0.91	5.2±1.22	0.7±0.42**[c]

* Different from vehicle ($P<0.05$)
** Different from vehicle ($P<0.01$)

[a] Mean±SE
[b] McNemar's test
[c] Wilcoxon matched pairs signed-ranks test

Sociosexual interaction

When the frequency of sociosexual interactions was analysed with the MANOVA, significant main effects of stimulus animal and dose were found, as well as a significant interaction between stimulus animal and dose. The durations of sociosexual interactions were also found to be different between the stimulus animals, between doses and the interaction stimulus animal-dose was also significant.

Table 3. Sexual behavior in male rats treated with s-baclofen 20 min before observation. $N=18$ at each dose

Behavior parameter	Vehicle	s-Baclofen 10.0 mg/kg
Mount percentage	88	94
Intromission percentage	72	88
Ejaculation percentage	50	38
Number of mounts[a]	3.0±0.73	3.1±1.23
Number of intromissions[a]	6.6±0.75	6.7±0.99

[a] Mean±SE

Table 4. MANOVA statistics obtained in the experiment on sociosexual interactions after treatment with R, s-baclofen

	Pillais V	DF	F	P
Main effects				
Frequency				
Stimulus animal	0.998	8,15	34.48	<0.001
Dose	1.042	16,76	5.17	<0.001
Stimulus animal – dose	0.860	16,76	3.58	<0.001
Duration				
Stimulus animal	0.944	6,17	48.06	<0.001
Dose	0.809	12,80	4.56	<0.001
Stimulus animal – dose	0.648	12,80	3.19	<0.001
Simple main effects				
Frequency				
Stimulus animal				
Vehicle	0.970	8,15	60.80	<0.001
Baclofen 2.5 mg/kg	0.859	8,15	11.50	<0.001
Baclofen 5 mg/kg	0.636	8,15	3.27	0.023
Dose				
Castrated male	0.605	16,76	2.06	0.019
Receptive female	1.192	16,76	7.00	<0.001
Duration				
Stimulus animal				
Vehicle	0.947	6,17	44.89	<0.001
Baclofen 2.5 mg/kg	0.805	6,17	11.70	<0.001
Baclofen 5 mg/kg	0.812	6,17	12.24	<0.001
Dose				
Castrated male	0.371	12,80	1.52	0.134
Receptive female	0.980	12,80	6.40	<0.001

Therefore, the simple main effects were analysed. MANOVA statistics are summarized in Table 4, and are therefore not mentioned in the text.

Analysing simple main effects of stimulus animal, a significant difference in frequency and duration of sociosexual interactions was found between the animals placed with a receptive female and those placed with a castrated male when the control treatment was administered (Tables 5 and 6). ANOVAs showed that the duration of rearing was shorter for the animals placed with a receptive female than for those placed with a castrated male. A similar difference in frequency and duration of grooming partner was found. In contrast, a higher frequency and longer duration of resting and pursuit was observed when the males were placed with receptive females. Similarly, a higher frequency of self-grooming was observed when the animals were placed with a receptive female than when placed with a castrated male.

When a dose of 2.5 mg/kg R, s-baclofen was administered, the omnibus test showed that frequencies and durations of sociosexual contacts were different between stimulus animals. ANOVAs showed that the frequency and duration of grooming partner were reduced when the animals were observed with a receptive female in comparison to when they were observed with a castrated male. In contrast, frequency and duration of pursuit were increased when the animals were placed with a receptive female (Tables 5 and 6).

When R, s-baclofen (5 mg/kg) was administered, the omnibus test again showed that frequencies as well as durations of sociosexual contacts were different between the stimulus animals. ANOVAs showed that the parameters that reflect some form of sexual interactions were much reduced after drug treatment when the stimulus animal was a receptive female. In fact, the only difference between stimulus animals that remained was a higher frequency and duration of pursuit for the males placed with a receptive female (Tables 5 and 6). Similarly, resting duration increased after drug treatment when the animals were placed with a receptive female but not when they were placed with a castrated male (Table 6). These results indicate that R, s-baclofen 5 mg/kg makes the behavior of the male with a receptive female very similar to the behavior of the male with a castrated male.

When analysing for simple main effects of dose, keeping stimulus animal constant, baclofen had a significant effect on the frequency of sociosexual interaction when the stimulus animal was a castrated male. However, pairwise comparisons between each baclofen dose and vehicle showed that neither of these doses was significantly different from control treatment [$T^2=0.303$, $F(8,15)=0.81$, $P=0.599$ when baclofen 2.5 mg/kg is compared to vehicle; $T^2=0.559$, $F(8,15)=2.37$, $P=0.071$ when baclofen 5 mg/kg is compared to vehicle]. No effect was found in duration of sociosexual interactions when the stimulus animal was a castrated male.

When the stimulus animal was a receptive female a significant effect was found in the frequency and duration of sociosexual interactions. Multivariate pairwise comparisons between each baclofen dose and vehicle showed that baclofen 2.5 mg/kg and 5 mg/kg were significantly different from vehicle [frequencies, $T^2=10.053$, $F(8,15)=18.85$, $P<0.001$; durations $T^2=0.701$, $F(6,17)=6.66$, $P=0.001$ for R, s-baclofen 2.5 mg/kg; frequencies $T^2=11.582$, $F(8,15)=21.71$, $P<0.001$; durations $T^2=2.242$, $F(6,17)=6.35$, $P=0.001$ for R, s-baclofen 5 mg/kg).

Univariate pairwise comparisons showed that baclofen 2.5 mg/kg reduced the frequency and duration of pursuit as well as the frequency of resting (Tables 4 and 5). When a dose of 5 mg/kg of baclofen was administered, the frequency and duration of self grooming and pursuit were reduced. Similarly, the frequency of rearing was reduced after baclofen treatment (5 mg/kg). In contrast, the duration of resting increased in animals treated with 5 mg/kg R, s-baclofen. It can be seen that this dose inhibits behaviors related to sexual interactions (self-grooming and pursuit).

The significant effects of baclofen on socio-sexual interactions are summarized in Fig. 1.

Table 5. Frequency of sociosexual interactions in animals observed with a receptive female or a castrated male after treatment with R, s-baclofen (mean ± SE). $N = 12$ at each dose

	Vehicle		R, s-Baclofen (2.5 mg/kg)		R, s-Baclofen (5 mg/kg)	
	Receptive female	Castrated male	Receptive female	Castrated male	Receptive female	Castrated male
Sniffing	24.0 ± 3.02	32.3 ± 3.58	29.3 ± 2.09	27.3 ± 2.74	17.2 ± 1.84	25.8 ± 3.32
Self-grooming	31.5 ± 1.67*	19.3 ± 1.58	27.8 ± 2.67	17.0 ± 2.92	9.5 ± 3.07^{+}	13.7 ± 2.99
Rearing	11.6 ± 1.31	18.5 ± 3.56	11.5 ± 1.10	15.2 ± 2.69	2.3 ± 0.91^{+}	9.1 ± 2.19
Resting	7.7 ± 1.95*	–	0.9 ± 0.47^{+}	0.3 ± 0.33	10.6 ± 1.53	6.3 ± 3.05
Grooming partner	2.4 ± 0.77*	12.1 ± 1.32	3.4 ± 0.84*	8.9 ± 1.59	2.9 ± 1.16	8.1 ± 2.34
Genital exploration	1.2 ± 0.49	6.1 ± 2.34	2.1 ± 1.52	2.3 ± 0.75	1.0 ± 0.51	1.8 ± 0.74
Pursuit	15.1 ± 0.80*	0.3 ± 0.25	10.2 ± 1.55*$^{+}$	–	4.2 ± 1.35*$^{+}$	0.1 ± 0.08
Incomplete mount	3.2 ± 1.15	1.2 ± 0.57	3.0 ± 0.83	1.7 ± 0.55	1.2 ± 0.60	0.8 ± 0.30

* Different from castrated male, same dose, $P < 0.05$
$^{+}$ Different from vehicle in the same testing situation, $P < 0.05$

Table 6. Duration of sociosexual interaction in animals observed with a receptive female or a castrated male after treatment with R, s-baclofen (mean ± SE). $N = 12$ at each dose

	Vehicle		R, s-Baclofen (2.5 mg/kg)		R, s-baclofen (5 mg/kg)	
	Receptive female	Castrated male	Receptive female	Castrated male	Receptive female	Castrated male
Sniffing	145.0 ± 17.68	201.6 ± 21.30	180.0 ± 23.48	201.6 ± 23.48	136.2 ± 22.43*	232.0 ± 23.31
Self grooming	230.7 ± 19.54	178.2 ± 33.72	243.4 ± 19.58	177.1 ± 31.91	101.7 ± 38.32^{+}	135.7 ± 31.25
Rearing	26.5 ± 3.52*	70.1 ± 10.73	42.6 ± 6.50	65.2 ± 9.37	4.9 ± 2.12	44.1 ± 14.05
Resting	32.9 ± 6.57*	–	10.1 ± 6.56	–	267.1 ± 44.79*$^{+}$	74.1 ± 30.71
Grooming partner	2.9 ± 1.13*	54.3 ± 8.49	6.9 ± 1.74*	51.2 ± 10.48	15.6 ± 5.87	29.2 ± 7.87
Pursuit	34.5 ± 4.04*	0.4 ± 0.42	20.7 ± 4.60*$^{+}$	–	11.1 ± 3.57*$^{+}$	–

* Different from castrated male, same dose, $P < 0.05$
$^{+}$ Different from vehicle in the same testing situation, $P < 0.05$

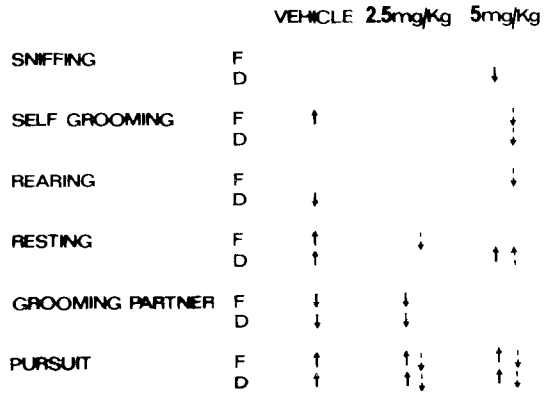

Fig. 1. Summary of the significant effects on sociosexual interactions obtained with different doses of R, s-baclofen or with different stimulus animals. The *solid arrows* indicate differences between stimulus animals and the *dotted arrows* differences between vehicle and baclofen when the animals were placed with a receptive female. ↑ increase ↓ decrease *F*, frequency; *D*, duration

With s-baclofen 10 mg/kg a significant main effect of stimulus animal was found [frequencies, Pillai's $V = 0.710$, $F(8,14) = 4.30$, $P = 0.009$; durations, Pillai's $V = 0.737$, $F(7,15) = 6.01$, $P = 0.002$). The frequency and duration of sniffing, rearing and grooming partner were reduced when the animals were placed with a receptive female in comparison to when they were placed with a castrated male. In contrast, a higher frequency and duration of pursuit was observed when the animals were placed with a receptive female. When the sociosexual interactions were analysed for the main effect of dose, no effect was found neither for frequencies [Pillai's $V = 0.296$, $F(8,14) = 0.73$, $P = 0.659$] nor for durations [Pillai's $V = 0.332$, $F(7,15) = 1.06$, $P = 0.429$]. Since a significant interaction between stimulus animal and dose was not found [frequencies, Pillai's $V = 0.258$, $F(8,14) = 0.60$, $P = 0.756$; durations, Pillai's $V = 0.321$, $F(7,15) = 1.01$, $P = 0.459$], no further analyses were done (data not shown). As in sexual behavior, s-baclofen is completely inactive.

Locomotor activity

The effects of the drugs on locomotor activity are shown in Fig. 2. Racemic baclofen and the R-enantiomer produced a dose-dependent reduction in locomotion. The s-enantiomer lacked effect even when a dose of 50 mg/kg was used. As in sexual behavior, it appears that the R-enantiomer is about twice as active as racemic baclofen, and the s-enantiomer seems to be completely inactive.

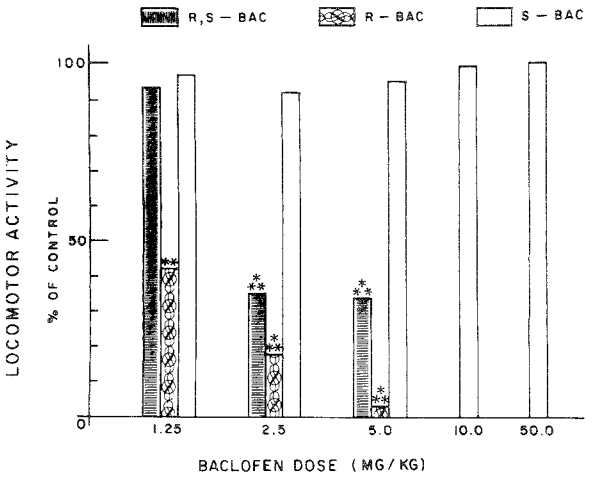

Fig. 2. Locomotor activity in male rats treated with varying doses of racemic baclofen and baclofen enantiomers ($N=10$ at each dose). ** Different from vehicle ($P<0.01$); *** Different from vehicle ($P<0.001$), t-test

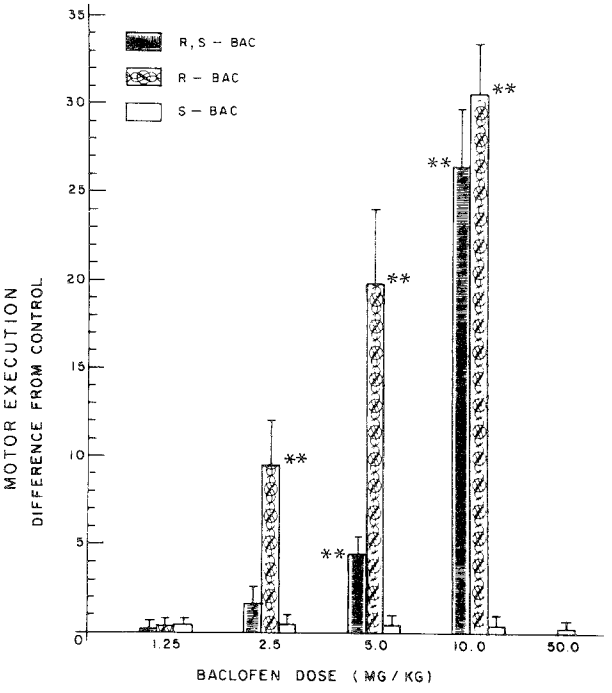

Fig. 3. Motor execution in male rats treated with varying doses of racemic baclofen and baclofen enantiomers ($N=10$ at each dose). ** Different from vehicle ($P<0.01$), Wilcoxon matched-pairs signed-ranks test

Motor execution

The lowest dose of R-baclofen with a significant effect was 2.5 mg/kg, whereas R, S-baclofen had to be administered in a dose of 5 mg/kg in order to obtain a similar effect. S-baclofen had no effect at the highest dose used, 50 mg/kg. Again it appears that R-baclofen is about twice as active as R, S-baclofen, and S-baclofen seems to be completely inactive. Data are summarized in Fig. 3.

Discussion

The data obtained in the present studies with baclofen enantiomers further support the hypothesis previously presented that the GABA-B receptor is involved in the control of behavioral functions (Agmo and Giordano 1985; Agmo and Paredes 1985). Furthermore, the data also show that the stereospecificity demonstrated in receptor binding studies (Olpe et al. 1978; Hill and Bowery 1981; Henry 1982; Bowery et al. 1983; Drew 1984; Haas et al. 1985) is also obtained with regard to the behavioral effects of baclofen. R-Baclofen reduced sexual behavior, locomotor activity and motor execution in relatively low doses, while S-baclofen was completely inactive even when a dose 40 times higher than the minimum effective dose of R-baclofen was used. A low dose of R-baclofen, 1.25 mg/kg, reduced locomotor activity by 58% while a high dose of S-baclofen, 50 mg/kg, did not have any significant effect. The R-enantiomer seems to be twice as active as the racemic baclofen. This can be clearly seen in all the observed behaviors in which twice the dose of R, S-baclofen is necessary to obtain an effect of similar magnitude to that of R-baclofen.

The data also show that the inhibition of sexual behavior produced by R and R, S-baclofen is similar to that produced by other GABAergic drugs (Agmo and Paredes 1985). That is, baclofen reduced the probability that the animal would engage in copulatory behavior (mount and intromission percentage and consequently mean number of mounts and intromissions). However, animals displaying sexual behavior showed a normal copulatory pattern. This might indicate that changes in GABAergic neurotransmission are incapable of modifying the mechanisms underlying that behavior once activated.

According to Hlinak (1986), heterosexual interaction can be divided in at least three phases. The precopulatory phase includes crawling over the female, grooming partner and pursuit of the female. The copulatory phase is the actual performance of sexual intercourse with the female. The postcopulatory phase is characterized by grooming and resting coinciding with the postejaculatory interval. As soon as the male begins to copulate, the occurrence of precopulatory behaviors is reduced. However, they may reappear between copulatory series (Hlinak 1986).

Baclofen 2.5 and 5 mg/kg significantly reduced behavioral patterns belonging to the precopulatory phase. The frequency and duration of pursuit was reduced with these doses when the animals were observed with a receptive female. However, only the higher dose, that is 5 mg/kg, inhibited sexual behavior. These results seems to demonstrate that although both doses of R, S-baclofen reduced the frequency of pursuit, the reduction needs to be considerable in order to inhibit copulatory behavior (as occurred with 5 mg/kg). Furthermore, baclofen 5 mg/kg also inhibited behaviors related to sexual interactions (self-grooming and rearing), whereas baclofen 2.5 mg/kg did not affect these behaviors.

The inhibitory effects seen with baclofen in sociosexual interactions were present only when the animals were observed with a receptive female. This suggests that the inhibitory effects correspond to sexual and not to social interactions.

A crucial question is whether baclofen acts directly on neural circuits involved in the control of sociosexual behavior, or whether that behavior is affected only because of a reduction in motility or an impairment of motor execution. It has previously been shown that the inhibitory effects of GABAergic drugs on sexual behavior in the male rat are probably independent of their effects on locomotor ac-

tivity (Agmo and Paredes 1985). In a similar study, no correlation could be found between the effects of GABA transaminase inhibitors on locomotor activity and their effects on sexual behavior (Agmo et al. 1987). However, a high correlation was obtained between impairment of motor execution and inhibition of sexual behavior.

In the present experiments, doses of baclofen that produced a moderate inhibition of locomotor activity did not affect sexual behavior. R-Baclofen 1.25 mg/kg and R, S-baclofen 2.5 mg/kg reduced locomotor activity by more than 50% but did not have any effects on sexual behavior. In contrast, inhibition of sexual behavior was accompanied by impairment of motor execution. R-Baclofen 2.5 mg/kg and R, S-baclofen 5 mg/kg both reduced motor execution and inhibited sexual behavior. These results apparently support the previous observation that whenever motor execution is impaired, sexual behavior is also impaired. However, data obtained in the experiment of sociosexual interaction suggest an additional effect of baclofen on sexual motivation. If baclofen produced a nonspecific inhibition of sexual behavior related to an impairment of motor execution, this impairment should also affect social interactions. However, as previously mentioned, the inhibitory effects of baclofen were restricted only to sexual interactions, since no effect was observed in social interactions with a castrated male. Therefore, it can be suggested that baclofen specifically inhibits precopulatory behaviors and hence the initiation of sexual behavior. Furthermore, in a previous study (Agmo and Paredes 1985) it was found that baclofen, in doses without effects on motor execution, did inhibit copulatory activity in castrated rats maintained at a subnormal level of sexual behavior with weekly injections of low doses of testosterone propionate. This might indicate that in this species of animal the inhibitory effects of baclofen on sexual behavior are independent of motor deficiencies. Another possibility is that the inhibitory effects of baclofen on sexual behavior depend on the androgen level of the animal. These alternative explanations are at present the subject of further experiments.

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