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EFFECT OF THE SERINE-BORATE COMPLEX ON THE RELATIVE ABILITY OF LEUKOTRIENE C_4, D_4 AND E_4 TO INHIBIT LUNG AND BRAIN [^{3}H] LEUKOTRIENE D_4 AND [^{3}H]LEUKOTRIENE C_4 BINDING: DEMONSTRATION OF THE AGONISTS' POTENCY ORDER FOR LEUKOTRIENE D_4 AND LEUKOTRIENE C_4 RECEPTORS

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To define the potency order of the leukotrienes for inhibition of [^{3}H] leukotriene D_4 and [^{3}H]leukotriene C_4 binding, we investigated leukotriene C_4, D_4 and E_4 competition with and without the serine-borate complex in guinea pig lung and brain homogenates. Without it, the rank order of their potency for inhibition of lung [^{3}H]leukotriene D_4 or [^{3}H]leukotriene C_4 binding was leukotriene C_4 = leukotriene D_4 > leukotriene E_4. Presence of the complex altered the potency order for both competition studies: for the [^{3}H] leukotriene D_4 competition it was leukotriene D_4 > leukotriene E_4 = leukotriene C_4 and for the [^{3}H]leukotriene C_4 competition it was leukotriene C_4 >> leukotriene D_4 ≧ leukotriene E_4.

Using [^{3}H]LTD_4 and [^{3}H]LTC_4, we and Bruns et al.(1,2) and Pong et al.(3) have respectively demonstrated specific LTD_4 and LTC_4 receptor binding sites in guinea pig and rat lung homogenates. The apparent difference between the relative potencies for LTC_4, LTD_4 and LTE_4 to inhibit [^{3}H] LTD_4 and [^{3}H] LTC_4 binding suggests existence of distinct pulmonary LTD_4 and LTC_4 receptors. However, for the LTD_4 receptor studies(1,2), no attempt was made to prevent the possible conversion of LTC_4 to LTD_4 by a membrane-bound enzyme, γ-glutamyltranspeptidase (γ-GTP)(4,5). To define precisely the rank order of the potency for the LTs acting on LTD_4 and LTC_4 receptors, we set out to determine the relative ability of the LTs to inhibit [^{3}H] LTD_4 or [^{3}H] LTC_4 binding in the presence and absence of an enzyme inhibitor, the serine-borate complex (6), in guinea pig lung and brain homogenates.

METHODS

The LTs were a gift from Dr. J. Rokach (Merck Frosst Canada, Inc.). The serine and related compounds were purchased from Sigma Co. (St. Louis, Mo),

Abbreviations: LT, leukotriene; SBC, serine-borate complex; γ-GTP, γ-glutamyltranspeptidase.

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and sodium borate was from Fisher Scientific Co. (Fair Lawn, N.J.). (14,15-[^{3}H]LTD_4) (Specific activity = 40.3 Ci/mmol) and (14,15-[^{3}H]LTC_4) (35.7 Ci/mmol) were purchased from New England Nuclear Co. and stored under argon at -20°C. We prepared the LT stock solutions and determined the actual stock concentrations according to the procedures reported previously(1). Only corrected concentrations of the LTs are presented in this report. After completion of all experiments, the LTs remained 80-100% active. The serine-borate complex (SBC) consisted of a mixture of 5 mM L-serine and 10 mM sodium borate (6).

We prepared crude lung and brain membrane homogenates of Hartley female guinea pigs (1-month-old), performed binding assays and calculated binding data according to the previous methods (7) with slight modification (1). We used 9.5×10^{-7} M LTD_4 and 1.52×10^{-6} M LTC_4 to define non-specific [^{3}H]LTD_4 and [^{3}H]LTC_4 binding, respectively. The IC_{50} value is the concentration of the LT that reduces binding by 50%. All values are expressed as mean ± SE.

RESULTS AND DISCUSSION

L-serine or sodium borate alone slightly inhibited specific [^{3}H]LTD_4 and [^{3}H]LTC_4 binding and the mixture of the two only increased [^{3}H]LTC_4 binding in both lung and brain homogenates. To determine if SBC inhibits the γ-GTP activity, we assessed the effect of serine and related compounds on brain [^{3}H]LTC_4 binding in the presence of 10 mM borate. The reason for using the brain homogenate for this determination is because this homogenate contains exclusively [^{3}H]LTC_4 binding sites (unpublished data). We found that the presence of 5 mM L-serine and related compounds increased brain [^{3}H]LTC_4 binding by 19-171%. The demonstrated ability of these compounds to decrease the γ-GTP activity (6) correlated well with the amount of the increase in binding caused by these compounds (Fig. 1) and SBC had the greatest ability to produce both effects. These results indicate the effectiveness of SBC in preventing degradation of [^{3}H]LTC_4. In the brain homogenate, the relative ability of the LTs in inhibiting [^{3}H]LTC_4 binding in the presence of SBC was LTC_4 (IC_{50} = 2.92 ± 0.41 x 10^{-7} M, n=6) >> LTD_4 (25 ± 17 x 10^{-5} M, n=3) ≧ LTE_4 (> 24 x 10^{-5} M, n=3). There were no demonstrable [^{3}H]LTD_4 binding sites in the brain homogenate.

In the absence of SBC, the LTs effectively inhibited lung [^{3}H]LTD_4 binding and the rank order of potency for these effects was LTC_4 = LTD_4 > LTE_4 (Fig. 2A, Table 1). The order and their IC_{50} values are similar to those reported previously (1,2) when [^{3}H]LTD_4 was used as a ligand and the enzyme inhibitor was omitted from the assay.

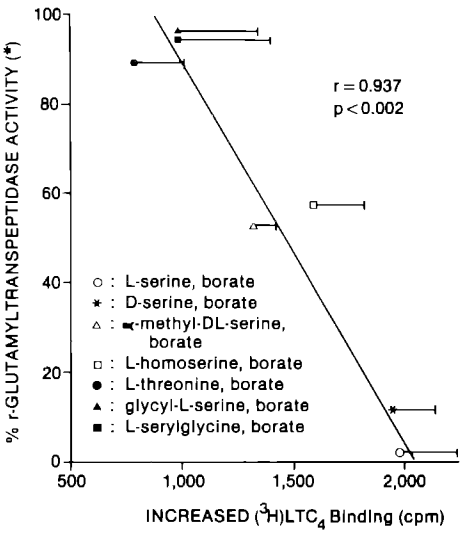

Fig. 1. Relationship between the effects of serine and related compounds on increased brain [^{3}H]LTC$_4$ binding and percent change of γ-GTP activity in the presence of 10 mM sodium borate. The brain homogenate (1.0 mg/ml) was incubated with 1.45 nM [^{3}H]LTC$_4$, with or without indicated compounds and in the presence and absence of 1.52 uM LTC$_4$. Specific [^{3}H]LTC$_4$ binding was 2481±947 cpm. Mean ± SE of 3 experiments. Percent γ-GTP activity (*) was from the result of Tate and Meister (Table 1, reference 6).

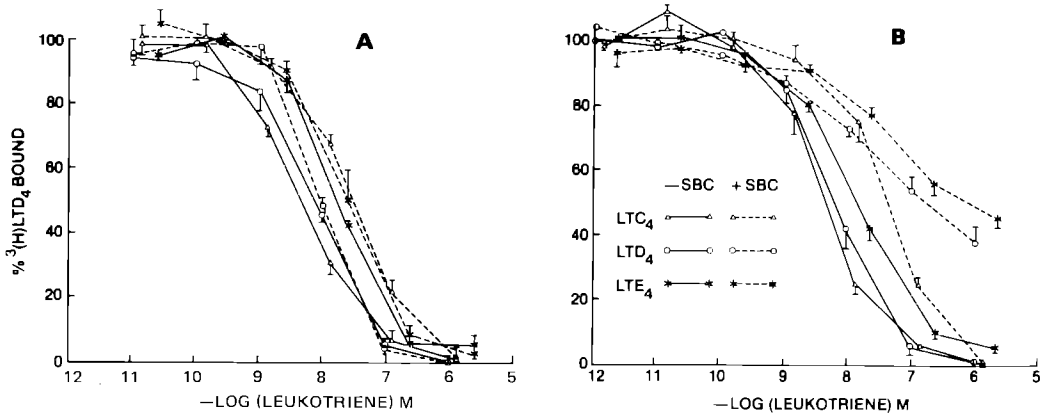

Fig. 2. Inhibition of [^{3}H]LTD$_4$ binding (A) and [^{3}H]LTC$_4$ binding (B) by the LTs in the presence and absence of the serine-borate complex (SBC).

A. The lung homogenate (1.0 mg/ml) was incubated with 0.79-1.42 nM [^{3}H]LTD$_4$ (9,500-17,200 cpm) in the presence and absence of 0.95 uM LTD$_4$, SBC and the listed agent. Control specific binding in the presence of SBC was 2,289±115 cpm and was 2,603±158 cpm (n=12) in the absence of SBC.

B. The lung homogenate (1.0 mg/ml) was incubated with 0.72-1.17 nM [^{3}H]LTC$_4$ (7,680-12,550 cpm) in the presence and absence of 1.52 uM LTC$_4$, SBC and the listed agent. Control specific binding in the presence of SBC was 2,445±170 cpm and was 2,148±136 cpm (n=12) in the absence of SBC. Mean ± SE of 4 experiments for each curve.

Table 1 IC_{50} values of LTC_4, LTD_4 and LTE_4 in the presence and absence of the serine-borate complex (SBC) in the guinea pig lung homogenate.

Variable			IC_{50} Value (M)+		
Group	Ligand	±SBC	LTC_4	LTD_4	LTE_4
I	[^{3}H] LTD_4	-SBC	$5.17\pm0.09\times10^{-9}$**	$6.40\pm0.47\times10^{-9}$**	$1.72\pm0.20\times10^{-8}$
II	[^{3}H] LTD_4	+SBC	$4.74\pm1.05\times10^{-8}$*	$7.68\pm1.09\times10^{-9}$	$2.84\pm0.84\times10^{-8}$
III	[^{3}H] LTC_4	-SBC	$4.74\pm0.73\times10^{-9}$**	$7.79\pm2.43\times10^{-9}$**	$1.63\pm0.23\times10^{-8}$
IV	[^{3}H] LTC_4	+SBC	$4.38\pm0.83\times10^{-8}$*	$3.70\pm1.67\times10^{-7}$	$1.26\pm0.83\times10^{-6}$

+ Each value represents the mean ± SE of 4 experiments.
For Group I: Comparison of IC_{50} values: $LTC_4 = LTD_4$, $LTC_4 < LTE_4$, $LTD_4 < LTE_4$.
Group II: $LTC_4 > LTD_4$, $LTC_4 = LTE_4$, $LTD_4 < LTE_4$.
Group III: $LTC_4 = LTD_4$, $LTC_4 < LTE_4$, $LTD_4 < LTE_4$.
Group IV: $LTC_4 < LTD_4$, $LTC_4 < LTE_4$, $LTD_4 = LTE_4$
Each comparison was tested by the Kruskal-Wallis analysis of variance by ranks and nonparametric multiple comparison (13). We consider the difference to be significant if $p < 0.05$.
* No difference between the two values (unpaired Student's t-test, $p > 0.05$).
** No difference among the four values (analysis of variance, $p > 0.05$).

The presence of SBC shifted the LTC_4/[^{3}H]LTD_4 competition curve to the right in a parallel manner, and neither LTD_4/[^{3}H]LTD_4 nor LTE_4/[^{3}H] LTD_4 competition curve was altered. Based on the IC_{50} values, LTD_4 was 3.7- and 6.2-fold more potent than LTE_4 and LTC_4, respectively, for inhibition of [^{3}H]LTD_4 binding (Group II, Table 1). Thus, the potency order following the SBC treatment was $LTD_4 > LTE_4 = LTC_4$.

In the absence of SBC, the LTs also effectively reduced lung [^{3}H]LTC_4 binding (Fig. 2B, Table 1) and, in fact, their relative ability to inhibit [^{3}H] LTC_4 binding was identical to their potency order in decreasing [^{3}H]LTD_4 binding (Group I vs. III, Table 1).

However, the presence of SBC significantly changed the ability of all three LTs and their potency order for inhibition of [^{3}H] LTC_4 binding. Addition of SBC shifted the LTC_4/[^{3}H]LTC_4 competition curve to the right and this effect was more pronounced on the LTD_4/[^{3}H] LTC_4 and LTE_4/ [^{3}H] LTC_4 competition curve (Fig. 2B). Moreover, the shift of the latter two was not in a parallel fashion. The Hill slope for the LTD_4/ [^{3}H] LTC_4 and

$LTE_4/[^3H]LTC_4$ competition in the absence of SBC was 0.99, 0.77 and in the presence of SBC was 0.39, 0.34 respectively. The IC_{50} value of each of the LTs was significantly increased by the addition of SBC (Group III vs. IV, Table 1): the increase was 9.2-fold for LTC_4, 47.5-fold for LTD_4 and 77.3-fold for LTE_4. Based on the IC_{50} values after the SBC treatment, LTC_4 was 8.4- and 28.8-fold more effective than LTD_4 and LTE_4 respectively in competing with $[^3H]LTC_4$ for lung binding sites (Group IV, Table 1). There is a slight but not statistical difference between the values of LTD_4 and LTE_4. Thus, the rank order of their potency in the presence of SBC for the LTC_4 binding sites is $LTC_4 >> LTD_4 \geqq LTE_4$.

We have demonstrated that the presence of SBC altered the potency order for the LTs and also the ability of some LTs to inhibit $[^3H]LTD_4$ and $[^3H]LTC_4$ binding in the lung homogenate. The SBC apparently prevents significant conversion of $[^3H]LTC_4$ or LTC_4 to $[^3H]LTD_4$ or LTD_4, respectively, by γ-GTP. In the absence of SBC, the IC_{50} value of the $LTC_4/[^3H]LTD_4$, $LTC_4/[^3H]LTC_4$ or $LTD_4/[^3H]LTC_4$ competition study did not differ significantly from that of the $LTD_4/[^3H]LTD_4$ competition result (Table 1). Although a decrease in the incubation temperature to 4°C reduces the conversion of LTC_4 to LTD_4 (3), the low temperature decreases specific $[^3H]LTD_4$ and $[^3H]LTC_4$ binding in both homogenates (data not shown). On the other hand, previous studies with the lung homogenate show only minimal conversion of $[^3H]LTD_4$ or LTD_4 to $[^3H]LTE_4$ or LTE_4, respectively (1,2).

By using SBC and LTs, we have defined the rank order of the agonists' potency for distinct LTD_4 and LTC_4 receptor binding sites: $LTD_4 > LTE_4 = LTC_4$ for the LTD_4 receptor and $LTC_4 >> LTD_4 \geqq LTE_4$ for the LTC_4 receptor. This potency order would help to identify pharmacologically the receptor binding sites and also can be used to correlate their relative functional responses at target organs. The effective interaction of LTC_4, LTD_4 and LTE_4 at $[^3H]LTD_4$ binding sites ($IC_{50} \leqq 5 \times 10^{-8}$ M) apparently reflects their effectiveness in stimulating the contraction of guinea pig lung parenchymal strips (1,8). The rank order of their potency in inhibiting $[^3H]LTD_4$ binding

is also compatible with the potency order obtained from several in vitro lung contraction experiments (9,10). These results suggest that the activation of the LTD_4 receptor would at least partly account for the airway contraction due to the LTs.

Recently, Hogaboom et al. (12) have showed that the potency order of the LTs for the lung LTD_4 receptor is $LTD_4 > LTE_4 > LTC_4$ and for the LTC_4 receptor is $LTC_4 >> LTE_4 > LTD_4$ (only one competition experiment for each LT is presented in Table II, reference 12). Both potency orders in their study appear to be different from our findings. Our potency order for lung LTC_4 receptor is similar to that for brain LTC_4 receptor.

Our results indicate that LTC_4 is equipotent ($IC_{50} = 4 \times 10^{-8}$ M) in acting on both LTD_4 and LTC_4 receptor binding sites, and LTD_4 and LTE_4 are less effective on the LTC_4 receptor site ($> 10^{-7}$ M). The latter finding is consistent with the results demonstrated in smooth muscle cell and rat lung homogenates (3,10). It appears that at the physiological concentration, LTC_4 may act on both receptors, whereas LTD_4 and LTE_4 would primarily interact with the LTD_4 receptor.

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