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Short communication

Enantiomers of clofibric acid analogs have opposite actions on rat skeletal muscle chloride channels

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Abstract: The S-(-) isomers of a series of clofibric acid analogs produced only a block of chloride conductance of rat skeletal muscle fibers with increasing concentrations until block was nearly complete. The R-(+) isomers, on the other hand, at low concentrations increased chloride conductance by as much as 9% to 39% and at higher concentrations decreased chloride conductance, but never by more than 27% of the control value. The actions of the enantiomeric pairs to either produce or inhibit myotonic excitability paralleled their ability to block or increase chloride conductance, respectively.

Key words: Chloride channel, Rat, Skeletal muscle, Stereoisomers, 2-(p-chloro-phenoxy)isobutyric acid, Clofibric acid, Myotonia

INTRODUCTION

We have previously shown that the S-(-) enantiomers of a series of 2-(p-chlorophenoxy)isobutyric acid (clofibric acid or CPIB) analogs blocked chloride conductance and induced myotonia in rat skeletal muscle fibers, whereas there was little or no chloride conductance block and no induction of myotonia by the R-(+) isomers at high concentrations [3]. We re-examined these effects in greater detail and have made the unexpected finding that the R-(+) isomers can produce substantial increases in chloride conductance at the low concentrations at which the corresponding S-(-) enantiomers have their threshold for decreasing the chloride conductance. The present study reports on this opposite action of the R-(+) and S-(-) enantiomers from four CPIB analogs which were compared by measurement of their effects on the resting membrane conductances and excitability of rat skeletal muscle fibers.

METHODS

Extensor digitorum longus (EDL) muscles were removed under anesthesia from male Wistar rats (180-220 g), installed in a muscle bath and superfused with physiological solution at 30°C. The test compounds were added to the bath from concentrated stock solutions as needed and the physiological solution was either normal or chloride-free (methyl sulfate substituted for chloride) ringers [5,6]. Measurements of chloride conductance, membrane potentials and membrane excitability were made as described previously [5,6] using standard intracellular microelectrode techniques. Estimates of chloride conductance were based on measurements of membrane resistance, Rm, in chloride-containing and chloride-free medium using a square pulse two microelectrode method. Single surface fibers were impaled by the two microelectrodes, one passed hyperpolarizing square wave constant current pulses (10-100 msec duration), the other measured the electrotonic potentials at two impalement sites from the current electrode, at about 0.05 mm and at about 0.5 to 1.0 mm. The current pulse was set to produce a hyperpolarization response of less than 20 mV at the close position. From the electronic potentials the values of Rm were calculated under each condition by standard cable analysis [5,6]. It was assumed that resting membrane conductance (1/Rm) in chloride-containing medium was the sum of potassium and chloride conductances, whereas the membrane conductance (1/Rm) in chloride-free medium was only potassium conductance. Chloride conductance is then the difference between these two measurements.

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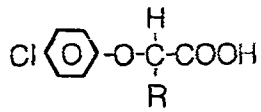
The four CPIB analogs examined in this study have the following structures:



ANALOG
2: R = CH_3- (methyl)
8: R = C_6H_5- (phenyl)
9: R = C_3H_7- (propyl)
10: R = C_6H_{13}- (hexyl)

These chiral compounds were synthesized, resolved and the absolute configurations determined following the procedures outlined previously [3]. The numbers designating each analog are consistent with this previous system. Racemates were also available.

RESULTS

Figures 1A and 1B show the effects of different concentrations of both enantiomers and the racemic mixtures of analog 2 (methyl derivative) and analog 8 (phenyl derivative) on the chloride conductance, respectively. The most striking effect is the remarkable increase in conductance produced in the concentration range below 10 μM by both R-(+) enantiomers. This conductance increase has never before been reported for any of the numerous carboxylic acids which affect skeletal muscle chloride conductance [4]. All four of the R-(+) enantiomers in this series showed this effect. Peak increases of 17% and 27% were produced by analogs 2 and 8, respectively, as shown in Figure 1, and peak increases of 39% and 9% by analogs 9 and 10, respectively, which are not shown. The S-(-) enantiomers produced only a block of chloride conductance at all concentrations, which is what is seen with achiral compounds such as anthracene-9-carboxylic acid [10] or the racemic mix-

tures of CPIB analogs shown in Figure 1 and previously [3]. In addition to the compounds in this paper we have examined several other S-(-) enantiomers and racemates of CPIB analogs in unpublished studies and have seen only a chloride conductance block. In the four enantiomeric pairs we report

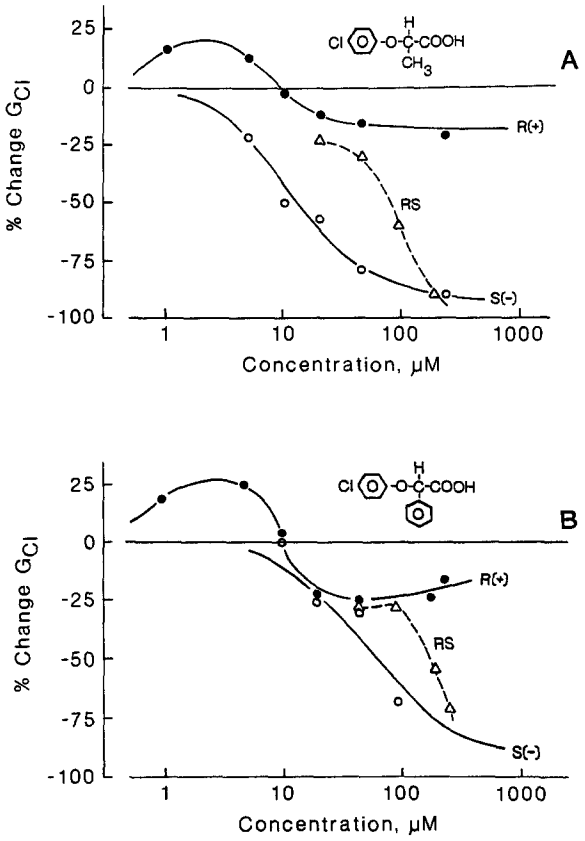


Fig. 1. Changes in resting chloride conductance Gcl produced by different concentrations of the R-(+), S-(-) and RS (racemic) forms of two CPIB analogs. (A) Analog 2, the methyl derivative. (B) Analog 8, the phenyl derivative.

here, the concentration of the R-(+) enantiomer which produced the peak increase was always lower than the IC50 of the corresponding S-(-) enantiomer which decreased chloride conductance. It can further be seen for the R-(+) enantiomers of analogs 2 and 8 in Figure 1 that above the 10 μM range the compounds produce a decrease in chloride conductance which tends to saturate at 22% to 26% of control. A similar fall by 27% to a plateau was seen for analog 9. Analog 10 was not tested higher than 10 μM; at this concentration there was a fall to 9% of control. The racemic mixtures caused only a fall in chloride conductance at any concentration. Again, as reported previously [3], the racemic mixtures shift the IC50 with respect to the S-(-) enantiomers by a factor exceeding two, suggesting antagonism of the blocking action of the S-(-) enantiomers by the R-(+) enantiomers. This antagonistic action is currently being investigated. Part of the antagonism could be from the opposite intrinsic actions of opening and closing chloride channels, and part from competition for the same receptor site by the complementary enantiomers.

A decrease in membrane chloride conductance in mammalian skeletal muscle fibers is capable of increasing membrane excitability, with an accompanying tendency to produce abnormal repetitive firing of action potentials known as myotonia [2]. Myotonic excitability then is a sensitive indirect measure of the status of chloride channels in response to agents which block, open or antagonize the block of the channels. Given in Table 1 the effects of the R-(+) enantiomers on excitability at concentrations where the maximum increase in conductance occurs. The R-(+) enantiomers shown here increased the chloride conductance by 9 to 39% but produced only minor changes in the excitability of normal fibers. In a computer simulation, increasing the normal chloride conductance by 30% increased the threshold current by about 15% [11], but this order of change could not be seen in Table 1 due to statistical variation. Thus, the R-(+) enantiomers appear to produce only a pure increase in chloride conductance. Latency is so short under these conditions that it is difficult to see a further shortening of this parameter; on the other hand, a lengthening of latency is a good indicator of increased excitability when chloride conductance is

Concentration, μM	Control	S-(-)-2 20	R-(+)-2 1	R-(+)-8 5	R-(+)-9 5	R-(+)-10 1
Resting Potential, -mV	76±2	70±2	77±2	78±2	73±1	66±5
Latency, mSec	6.2±0.8	45.5±2.5	6.1±0.6	6.5±0.7	4.8±0.8	4.8±2.4
Threshold Current, nA	128±12	65±5	129±13	151.8±22	121.3±17	123±33
Number of Spikes	6.1±0.3	18.0±1.1	5.8±0.5	6.9±1.1	6.5±1.0	8.5±1.5
Fibers/Preparations	28/12	15/3	13/3	10/5	4/2	2/1
Change in GCl, %	-------	-57	+17	+27	+39	+9

Table 1. Effects of R-(+) enantiomers on membrane excitability. Values are mean ± SEM. Concentrations of R-(+) enantiomers were those at which the maximum increase in chloride conductance occurred. For comparison S-(-)-2 was given at a concentration which gave an extreme myotonic excitability. Single fiber excitability was measured as the response to long (200 msec duration) depolarizing pulses delivered by the current electrode as recorded by a closely placed voltage electrode. The parameters are given here in successive rows from the top starting with the resting potential on impalement, the latency from the start of the current pulse to initiation of an action potential at threshold, the threshold current (rheobase), the number spikes (action potentials) obtained during the pulse when a current twice threshold is given.

decreased from normal [2], as is shown for comparison for the S-(-)-2 enantiomer. Only for R-(+)-9 were there significant changes in the parameters indicating decreased excitability. However, in all cases the R-(+) enantiomers were devoid of myotonic firing of action potentials.

DISCUSSION

The S-(-) and R-(+) enantiomers of this series of CPIB analogs show opposite effects on resting chloride conductance, the S-(-) always decreasing conductance and the R-(+) always increasing conductance at low concentrations and decreasing conductance by only a limited amount at high concentrations. The pharmacological property of enantiomers to have opposite effects on the probability of channel opening is not unique. The enantiomers of the BAY K 8644 [8,9] and the 202-791 [12] dihydropyridine (DHP) compounds also show this property on the calcium channel. In the case of the BAY K 8644 compound the (+) enantiomer blocks calcium channels and the (-) enantiomer, the so called "agonist", increases opening probability. The DHP and our CPIB enantiomers both act at approximately ten fold different concentrations for their respective effects.

The action of the R-(+) enantiomers of the CPIB analogs to increase chloride conductance occurs at lower concentrations than does the blocking effect of the S-(-) enantiomers. This fact could be important in our search for higher affinity ligands with which to purify the skeletal muscle chloride channel proteins and to eventually determine primary structures. Closely related compounds, the chiral phenoxy carboxylic acids, affect the auxin receptor of plants in a manner similar to that of our compounds on muscle. The R-(+) enantiomers that turn on chloride channels also act as agonists at the auxin receptor; whereas S-(-) enantiomers which block chloride conductance in muscle act as auxin inhibitors [1]. We have reported elsewhere that the R-(+) enantiomers of CPIB congenors block cholesterol synthesis more effectively, thus allowing control of the myotonia side effect [7].

Regarding the mechanism of the opposite agonistic actions enantiomers of CPIB analogs we can not offer a satisfactory explanation. The simplest situation might be to have a single species of channel with single sites at which the enantiomers compete; however, separate sites for each enantiomer on each channel or even two types of channels are also reasonable proposals, but there not enough evidence at this time to support any particular model.

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