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CALCIUM CHANNEL AGONIST AND ANTAGONIST EFFECTS OF THE STEREOISOMERS OF THE DIHYDROPYRIDINE 202-791

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The effects of the pure stereoisomers of the novel dihydropyridine 202-791 on voltage sensitive calcium channels in nerve and cardiac muscle were examined. The (-)-isomer blocked depolarization-induced uptake of $^{45}Ca^{2+}$ into NG108-15 neuroblastoma x glioma cells, blocked the depolarization-induced release of [^{3}H]-norepinephrine from PC12 cells and reduced the Vmax of the slow response action potential recorded from guinea pig papillary muscle. In contrast, the (+)-isomer enhanced these same processes. In papillary muscle, greater enhancement of the slow responses was observed at lower stimulation frequencies. Thus, the (-) and (+) stereoisomers of 202-791 can be shown to be calcium channel antagonist and agonist respectively.

Drugs and toxins have proven to be essential probes of the structure and function of receptors and ion channels. Owing to the importance of Ca^{2+} as a biological messenger, there is currently great interest in molecules such as channels that regulate the distribution of this ion across the cell membrane. It is likely that several types of voltage sensitive calcium channels (VSCC) exist (1). One major type of VSCC that is found in several cell types including nerve and muscle, is characterized by its sensitivity to dihydropyridines (DHP). Some DHP (antagonists) can block the flow of Ca^{2+} through the channel, whereas others (agonists) allow the channel to remain open for increased periods of time (2). These actions have been important in the elucidation of the mechanisms by which VSCC are normally regulated (2). The experiments presented here examine the activity

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of a novel DHP, 202-791 (3), on VSCC in nerve and cardiac muscle and demonstrate that its stereoisomers have opposite channel modulating properties.

MATERIALS AND METHODS

Drugs. 202-791 and its pure stereoisomers were the kind gift of Dr. R.P. Hof, Sandoz Ltd. Basel, Switzerland.

$^{45}Ca^{2+}$ Uptake Assay. Assays were carried out in differentiated NG108-15 cells. Cell culture and assay conditions were exactly as described previously (4), except that the assay media used were the same as for [^{3}H]-norepinephrine release assays (5).

[^{3}H]-Norepinephrine Release Assay. Assays were carried out using cultured PC12 pheochromocytoma cells. Culture and assay conditions were exactly as described previously (5), except where noted.

Cardiac Electrophysiology. Papillary muscles were isolated from right ventricles of guinea pigs (250-350gm, either sex). Slow response action potentials were recorded and analyzed as described previously (6).

RESULTS

We have previously demonstrated that several neuronal clonal cell lines possess VSCC that are sensitive to DHP (4,8,9). The effect of 202-791 on VSCC in the neuroblastoma x glioma hybrid cell line NG108-15 is illustrated in FIG. 1. It can be seen that raising the external [K^+] from 5 to 70mM caused an increase in the uptake of $^{45}Ca^{2+}$ by the cells. We have previously demonstrated that this increased uptake is via DHP sensitive VSCC (4,8,9). FIG. 1 shows that the two stereoisomers of 202-791 have opposite effects. When added in the presence of 70mM K^+, 10^{-7}M (-)-202-791 completely reduced the depolarization induced $^{45}Ca^{2+}$ uptake (FIG. 1B). In complete contrast to this, 10^{-6}M (+)-202-791 slightly enhanced $^{45}Ca^{2+}$ uptake in the presence of 5mM K^+ and greatly enhanced 70mM K^+ induced uptake, (FIG. 1A). These actions are reminiscent of those of VSCC antagonists such as nitrendipine and VSCC agonists such as BAY K 8644, respectively. Concentration-response relationships for these effects are illustrated in FIG. 2. (-)-202-791 blocked depolarization-induced

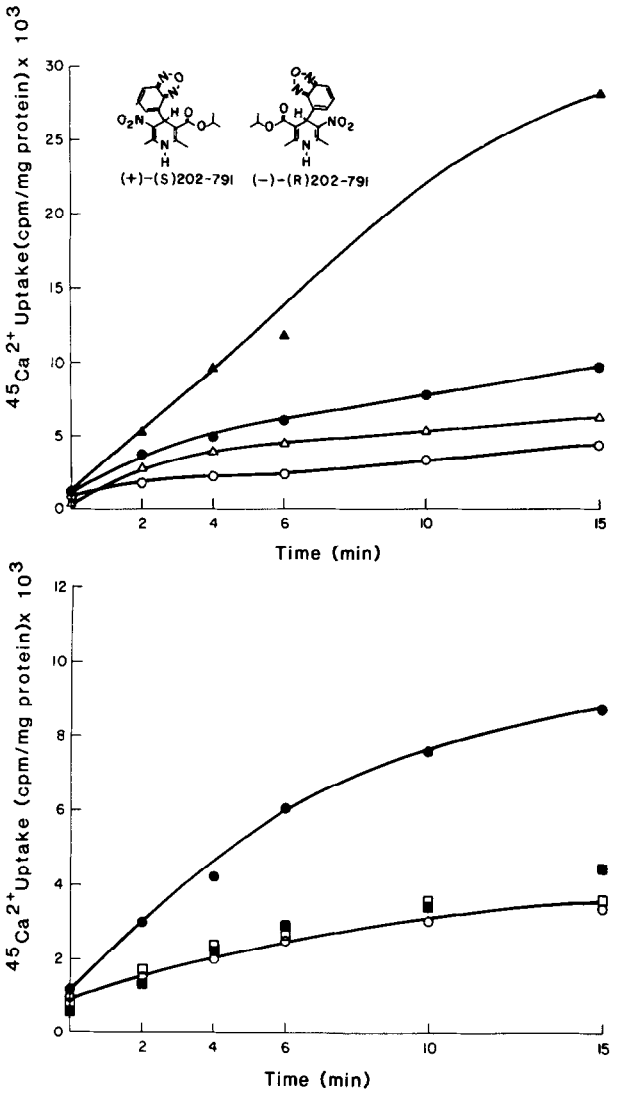

FIG. 1. Effects of isomers of 202-791 on depolarization-induced $^{45}Ca^{2+}$ uptake into NG108-15 cells. A. (○) 5mM K^+; (●) 70mM K^+; (△) 5mM K^+ + 10^{-6}M (+)-202-791; (▲) 70mM K^+ + 10^{-6}M (+) 202-791. B. (○) 5mM K^+; (●) 70mM K^+; (□) 5mM K^+ + 10^{-7}M (-) -202-791; (■) 70mM K^+ + 10^{-7}M (-)-202-791. Points are means of duplicate cultures. Results are representive of 2 separate experiments.

$^{45}Ca^{2+}$ uptake with an IC_{50} of $2x10^{-8}$M; (+)-202-791 enhanced depolarization-induced uptake with an EC_{50} of $7x10^{-8}$M. When the racemic mixture was tested, a blocking effect was obvserved but the "potency" of the racemate was less than that of the pure (-)-

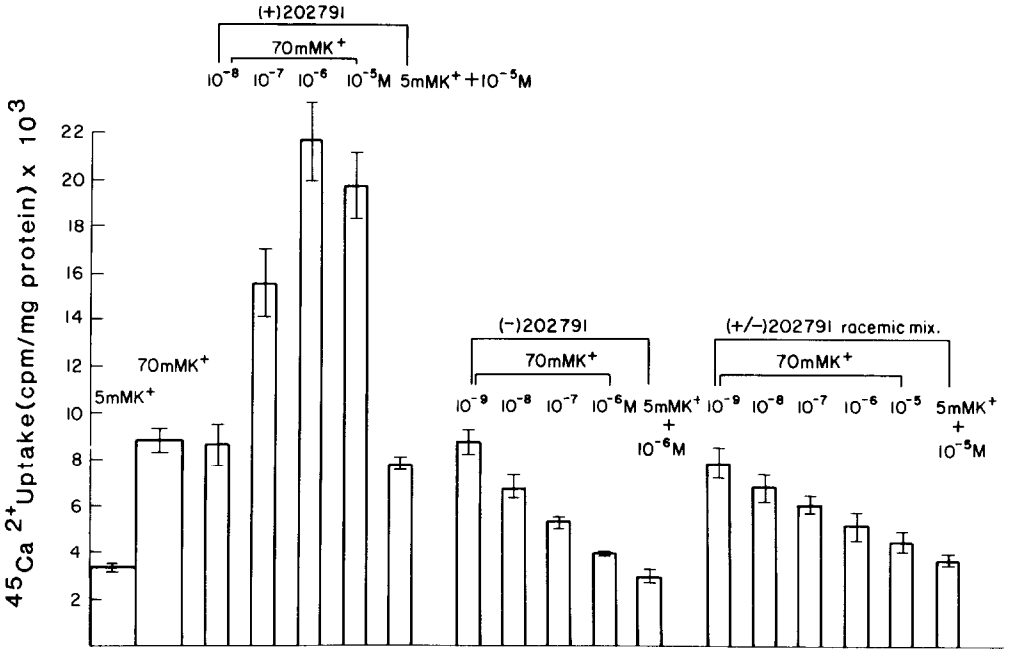

FIG. 2. Concentration-response relationships for the effects of 202-791 and its stereoisomers on depolarization induced $^{45}Ca^{2+}$ uptake into NG108-18 cells. Points are means SEM, n = 4.

isomer. This is consistent with the competitive interaction previously demonstrated for DHP agonists and antagonists (4).

In PC12 pheochromocytoma cells, DHP sensitive VSCC provide Ca^{2+} for the depolarization-induced release of norepinephrine (5). FIG. 3 shows that the two isomers of 202-791 also have disparate effects on VSCC in these cells. Increasing the external [K^+] provoked increasing release of [^3H]-norepinephrine from preloaded cells. This reached a maximum at approximately 50mM external K^+. This depolarization-induced release of transmitter is almost completely blocked by 10^{-7}M (-)-202-791. (+)-202-791 enhanced evoked transmitter release in a concentration dependent fashion. Interestingly, this enhancement was greater at lower external [K^+] and somewhat reduced at higher concentrations.

The isomers of 202-791 also had typical agonist and antagonist effects on VSCC in cardiac muscle (6,7). Slow response

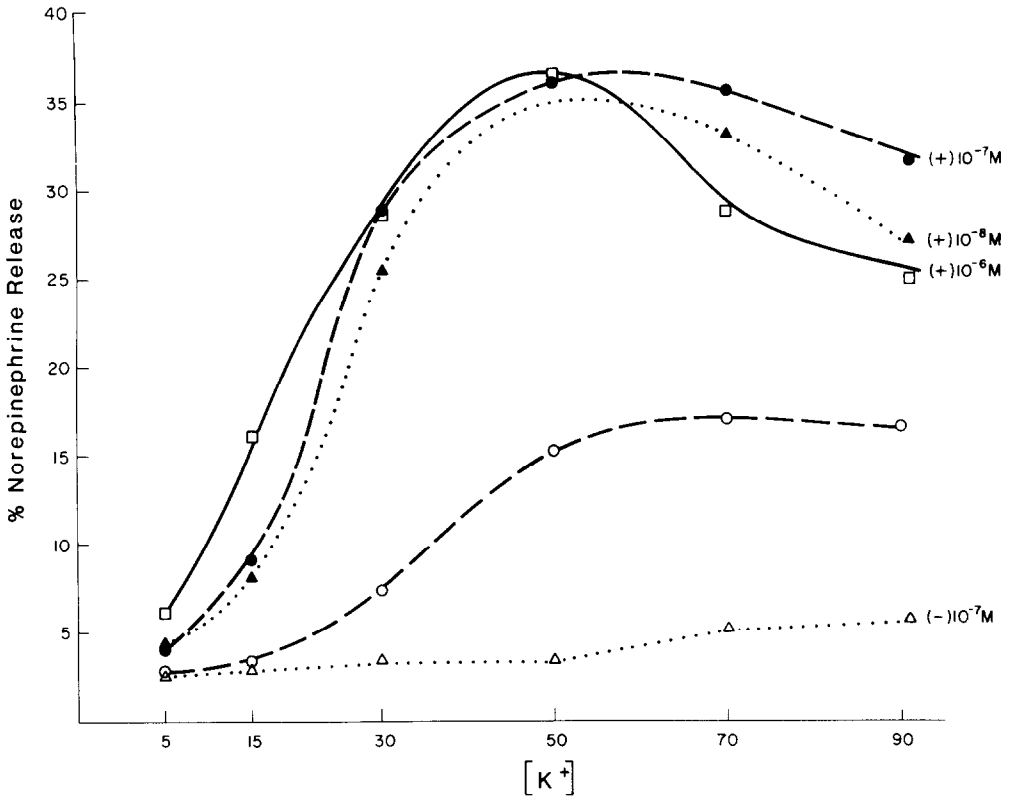

FIG. 3. Effects of the stereoisomers of 202-791 on depolarization induced release of [^{3}H]-norepinephrine from PC12 cells. Points are means of duplicate cultures. Media used is described in (5); variations in K^+ concentrations were matched by reciprocal changes in choline$^+$ concentrations.

action potentials in papillary muscle are dependent upon the movement of Ca^{2+} through DHP sensitive VSCC in this tissue (7). FIG. 4 shows that the maximum rate of rise of the action potential ($\dot{V}max$) was reduced by (-)-202-791 in a concentration-dependent fashion. Likewise, racemic 202-791 inhibited $\dot{V}max$ in a concentration-dependent manner, but it required higher concentrations for this effect than the pure (-)-isomer. In addition, the inhibitory effect of both racemic and (-)-202-791 was enhanced at higher rates of stimulation. Moreover, it can be seen that $\dot{V}max$ was enhanced by (+)-202-791. As previously demonstrated with CGP 28 392 (6), this enhancement was greater at low rates of stimulation and considerably decreased at higher rates. At higher

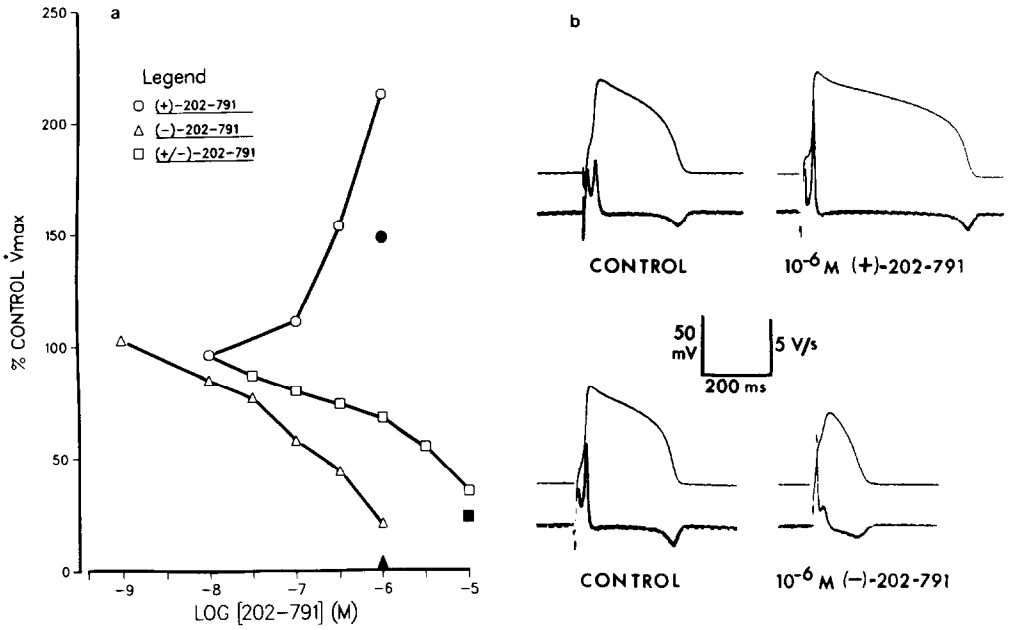

Fig. 4. Concentration-dependent effects of 202-791 on Vmax of slow response action potentials recorded from guinea pig papillary muscle. Panel (a) shows Vmax (% of control) at a stimulation rate of 12/min (open symbols) for different concentrations of (+)-202-791, (○), n = 3; (-)-202-791 (△), n = 3; and (+/-) -202-791 (□), n = 2. Closed symbols represent Vmax at a stimulation rate of 200/min for the highest concentration of each compound tested. Panel (b) shows slow response action potentials (upper traces) and dV/dt (lower traces) during control and after addition of 10^{-6}M of (+)-202-791 and (-)-202-791 (different preparations, stimulation rate = 12/min).

concentrations of (+)-202-791, ($>10^{-6}$M) the tissue became spontaneously active.

DISCUSSION

Although the action of DHP VSCC antagonists has been recognized for several years, the action of VSCC agonists is a recently discovered phenomenon (See 2). Indeed, careful analysis shows that most, if not all, known DHP's exhibit elements of both types of activity. For example, classical VSCC antagonists such as nifedipine can be shown to possess some agonist activity (10). Moreover, the newer agonists such as BAY K 8644 and CGP 28 392 can clearly show antagonist activity when examined under appropriate conditions (4,6,10). In smooth muscle, the racemic mix-

ture of 202-791 shows both kinds of activity (3). The present observations using pure stereoisomers of 202-791, as well as others using smooth muscle (3), raise an interesting possibility. It is clear that the two isomers of this compound act as VSCC agonist or antagonist. Thus, it is possible that the mixed properties previously observed with racemic drug mixtures are due to the presence of two compounds with disparate activities rather than being intrinsic properties of the same molecule. The relative ability of a racemate to act as an agonist or an antagonist may depend on the relative properties of its two isomers such as their affinities, as well as other factors. Is it the case that the stereoisomers of 202-791 and of other DHP, are in fact pure agonists or antagonists? It is difficult to give a definitive answer to this question from the data obtained so far. However, it should be pointed out that the pattern of activity of (+)-202-791 in guinea pig papillary muscle is reminiscent of that of (±) CGP 28 392 (6). We previously demonstrated that under appropriate conditions (27mM external K^+ and high stimulation rates) this latter compound could actually act as an antagonist. In the present study (+)-202-791 is clearly less effective at higher stimulation rates. Unfortunately, we were prevented from using increasingly depolarized conditions or higher drug concentrations due to the spontaneous activity induced by the drug under such circumstances. Thus we were unable to judge, using the present paradigm, whether (+)-202-791 could actually be made to demonstrate antagonist activity. Further electrophysiological studies, such as those previously carried out with BAY K 8644 will be necessary in order to finally decide this point (9).

It has been demonstrated in several studies that DHP VSCC channel agonists and antagonists interact in a competitive fashion (4). This implies an interaction at the same channel site.

The structural requirements determining agonist and antagonist activity subsequent to binding are extremely subtle. Indeed, as demonstrated with the isomers of 202-791, this extends to differences solely in the configuration of two substances. Such disparate actions of two enantiomers are extremely unusual. Clearly, these two substances should be important probes of VSCC structure and function.

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