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The Difference Between Nicardipine and Its Enantiomers on Inhibiting Vasoconstriction of Isolated Rabbit Thoracic Artery

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The present study was designed to study the difference effects between nicardipine and its two enantiomers on thoracic artery of rabbit. A high-performance liquid chromatographic method was used to prepare the two enantiomers of nicardipine. The thoracic artery of rabbit was removed. The vessels were cut into 3 mm in width and 15 mm in length spiral strips and immersed into tissue baths. The concentration-response curves of nicardipine and its enantiomers were obtained by cumulative administration of the vasoconstrictors. Nicardipine and the enantiomers could shift the dose-response curves of NE, KCl or $CaCl_2$ to right in a nonparallel manner and decrease the maximum effective in a concentration-depended manner, respectively. The pD_2' value of *R*-(-)-nicardipine showed significantly effective than that of nicardipine and *S*-(+)-nicardipine ($P<0.01$). There was not obviouse difference between the pD_2' value of nicardipine and *S*-(+)-nicardipine ($P>0.05$). The results demonstrate that the stereoselective interaction between *R*-(-)-nicardipine and L-calcium channel receptor is more stronger than that of *S*-(+)-nicardipine.

Key words: Nicardipine, Enantiomers, Vasoconstriction, Stereoselectivity

INTRODUCTION

Nicardipine hydrochloride, 2-(*N*-benzyl-*N*-methylamino) ethyl methyl 1,4-dihydro-2,6-dimethyl-4(*m*-nitrophenyl)-3,5-pyridinedecarboxylate monohydrochlorode (Fig. 1), is one of the 1,4-dihydropyridiane Ca^{2+} channel antagonists. It can selectively act on the L-type calcium channel and efficiently reduce Ca^{2+} inflow. By this mechanism, it can relax the smooth muscle of arteries and it been used for the treatment of hypertension and cerebrovascular diseases (Terai *et al.*, 1981). As is common knowledge, there are two enantiomers of this chemical because it contains an asymmetric carbon at position 4 of the dihydropridine ring. Nicardipine enantiomers have shown different vasodilator and hypotensive effects in dogs (Takenaka *et al.*, 1982) and rats (Brisac *et al.*, 1988). There were stereoselective pharmacokinetics (Tsukasa *et al.*, 1997) and different tissue distributions (Wang *et al.*, 2003) demonstrated between the enantiomers *in vivo*. The interaction and pharmacological effects of the nicardipine molecule with the L-type calcium channel

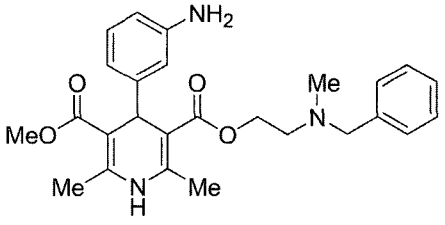

Fig. 1. Chemical structure of nicardipine[2-(*N*-benzyl-*N*-methylamino) ethyl methyl 1,4-dihydro-2,6-dimethyl-4(*m*-nitrophenyl)-3,5-pyridinedecarboxylate]. The monohydrochloride salt of nicardipine was used in the present study.

crossing the cell membrane happens within a three dimensional stereo-environment (Jiang *et al.*, 1996). Yet until now, this chemical has been synthesized and supplied as a racemic mixture. It is valuable to utilize and efficiently develop a single enentiomer for the clinical setting by investigating the differences between the enantiomers at the molecular level.

In this paper, the two enantiomers of nicardipine were prepared by using a high performance liquid chromatographic system equipped with a Chiralcel OJ column. The inhibitions of vasoconstriction for nicardipine and its enentiomers were investigated by arterial strip trial *in vitro*, respectively.

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MATERIALS AND METHODS

The experimental protocols for isolating rabbits descending thoracic arteries were approved by the animal ethics committees at Xi'an Jiaotong University.

Chemicals and materials

The Shimadzu 10A*vp* model high-performance liquid chromatographic system included an LC-10AT*vp* pump, a SPD-10A*vp* UV-detector and the ANASTAR workstation (Shimadzu, Tokyo, Japan). The muscular tension transducer and BL-410 model bio-function experimental system are produced by the Spaceflight Medical Engineering Institute (Beijing, China).

Racemic nicardipine [(±)-NCD] was obtained from Sigma (Sigma, USA), the HPLC-grade *n*-hexane and anhydrous ethanol were obtained from Fisher (Fisher, USA). The binodrenal injection solution (NE, 2 mg, 10 × 1 mL) was obtained from Wuhan Pharmaceutical Plant (Wuhan, China). Potassium chloride (KCl) and calcium chloride ($CaCl_2$) were obtained from Xi'an Chemical Reagent Plant (Xi'an, China). All the other chemicals were of analytical reagent grade unless otherwise indicated. The water was purified with an ion-exchange method and it was distilled before being used to prepare all the solutions.

Rabbits (male or female, 2.0-2.5 kg) were supplied by the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China).

Analytical methods

Preparation of nicardipine enantiomers

Nicardipine enantiomers were prepared by using HPLC with a semi-preparative Chiralcel OJ column (250 mm × 10 mm, I.D.) (Daicel, Tokyo, Japan). The chromatographic conditions were as follows. The mobile phase used *n*-hexane/anhydrous ethanol (85:15, v/v), the flow rate was 0.5 mL·min^{-1} and the detection wavelength was 236 nm. According to the literature (Wolfgang, 1995), Fischer *et al.* (1993), and Uno *et al.* (1997), the first peak with a 30 min retention time and the second peak with a 35 min retention time were *S*-(+) nicardipine [*S*-(+)-NCD] and *R*-(-) nicardipine [*R*-(-)-NCD] on the chromatogram, respectively. Under the same conditions, the racemic nicardipine standard solution was injected into the chromatographic system and the eluates were collected in sequence into standard a brown chemical bottles, respectively. The hexane in solution was evaporated under conditions of darkness and a nitrogen gas stream, and the ethanol solution containing each enantiomer was so obtained. The purities of enantiomers were analyzed by using the chiral HPLC method described above.

Preparation of the solutions

The analytical solutions of (±)-NCD and *S*-(+)-NCD and *R*-(-)-NCD were prepared by using the mixture solvent of tween 80/ethanol/physiological saline (1:1:8), and this was diluted with physiological saline to the required concentrations (Guan *et al.*, 1996), respectively.

Isolated rabbit aortic strip experiments

Young rabbits (body weight 2.0-2.5 kg) of either sex were decapitated under pentobarbital anesthesia. The descending thoracic artery was gently removed and immersed in cold buffer solution (see below), and the adhered tissue was dissected free. The vessels were then cut into spiral strips 3 mm in width and 15 mm in length. Each segment of aorta strip was mounted on two metal hooks, one of which was connected to a displacement device; the other was connected to a biofunction experimental system (Institute of Space Flight Medical-Engineering, China) for continuous recording of the isometric tension. The position of one hook could be changed by a movable unit, allowing fine adjustments of the vascular tension by varying the distance between the metal hooks.

The mounted specimens were immersed in temperature-controlled (37°C) tissue baths containing Krabs-Henseleit buffer solution with 118 mM NaCl, 4.7 mM KCl, 1.2 mM $KHPO_4$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$ and 11 mM glucose. The solution was continuously perfused with 5% CO_2 in O_2, giving the pH of 7.4.

A tension of 1.0 g was applied to the arterial segments, and they were allowed to stabilize at this level for 1.0 h. The contractile capacity of each strip was examined by exposing it to the norepinephrine (60 μM) in the standard buffer solution. Only when two reproducible contractions (>2.0 g) had been achieved were the aortic strips used for further study (variation <10%). The contractile effect of norepinephrine (NE) or KCl was studied only once in each strip in parallel experiments (with as many as 8 strips). Concentration-response curves were constructed by using the cumulative application of either NE or KCl as agonists until the maximum was reached with or without a varying concentration of (±)-NCD, *S*-(+)-NCD or *R*-(-)-NCD, which had been added 20 min earlier to the tissue bath. The (±)-NCD, *S*-(+)-NCD and *R*-(-)-NCD were tested only on separate sections of aorta strip. The contractile effect of $CaCl_2$ was tested by the following procedure: the aorta strips were placed in Ca^{2+}-free Krabs-Henseleit solution for 30 min, and then they were placed in Ca^{2+}-free Krabs-Henseleit solution containing KCl 40 mM for 20 min (Chen *et al.*, 1993; H. Gerhard *et al.*, 1997). The concentration depended effect of Ca^{2+} was then examined on the strips. We then washed the strips several times using Ca^{2+}-free Krabs-Henseleit solution. The tested drugs were added to examine their effects on the contraction induced by $CaCl_2$ and

cumulative concentration-response curves were obtained.

Statistical analysis

The data are expressed as mean ± SEM. The contractile responses in each segment are expressed as a percentage of the maximum-contraction induced in that segment by NE, KCl or $CaCl_2$ without the antagonists, respectively. In a given experiment E_{max} represented the maximum contraction induced by an agonist whereas the pD_2 value was calculated from the line between the concentrations above and below the midpoint of the concentration-response curve.

To evaluate statistical significance between the concentration-response values, the Students *t*-test was used. *P* values <0.05 were considered significant.

RESULTS

Separation of the enantiomers

The baseline separation with the resolution value (R_S) of 1.546 (more than 1.5) was obtained under the chromatographic conditions reported above. It was suitable for the preparation of each nicardipine eanatiomer with this chiral HPLC method (Fig. 2).The purity of each eanatiomer was measured by the same method. The results are shown in Fig. 2a and 2b, and the purity of *S*-(+)-NCD was 96.7% and that of *R*-(-)-NCD was 95.8% (Fig. 2a and 2b), respectively. It will be content with the Pharmacological studies.

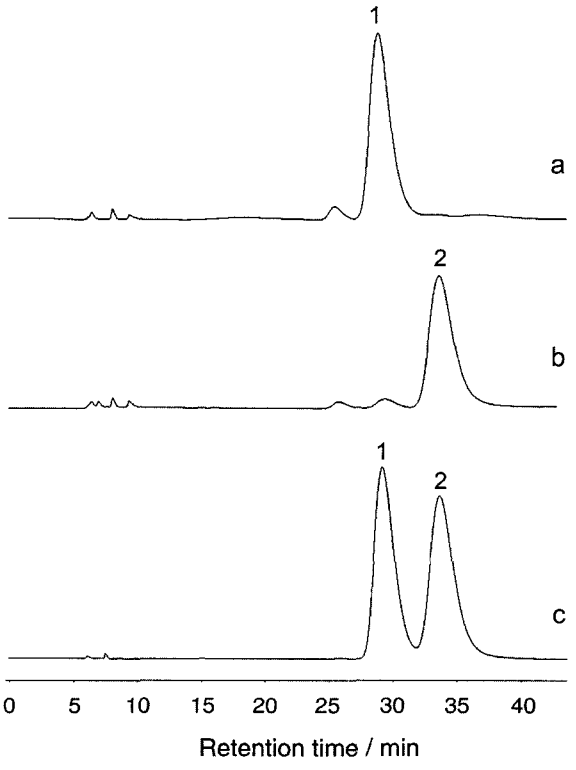

Fig. 2. Chromatograms of *S*-(+)-NCD (a), *R*-(-)-NCD (b) and (±)-NCD (c) on the Chiralcel OJ column. 1 is *S*-(+)-NCD, 2 is *R*-(-)-NCD.

Pharmacological studies

The NE, KCl and $CaCl_2$ induced a concentration dependent contractions on the aortic strips. The maximum responses to these three agents were set as 100%, respectively. Then the three different concentrations (0.1 mmol·L^{-1}, 0.5 mmol·L^{-1}, and 1.0 mmol·L^{-1}of each drug [(±)-NCD, *S*-(+)-NCD and *R*-(-)-NCD] were tested on the three kind of models, respectively. The results indicated that

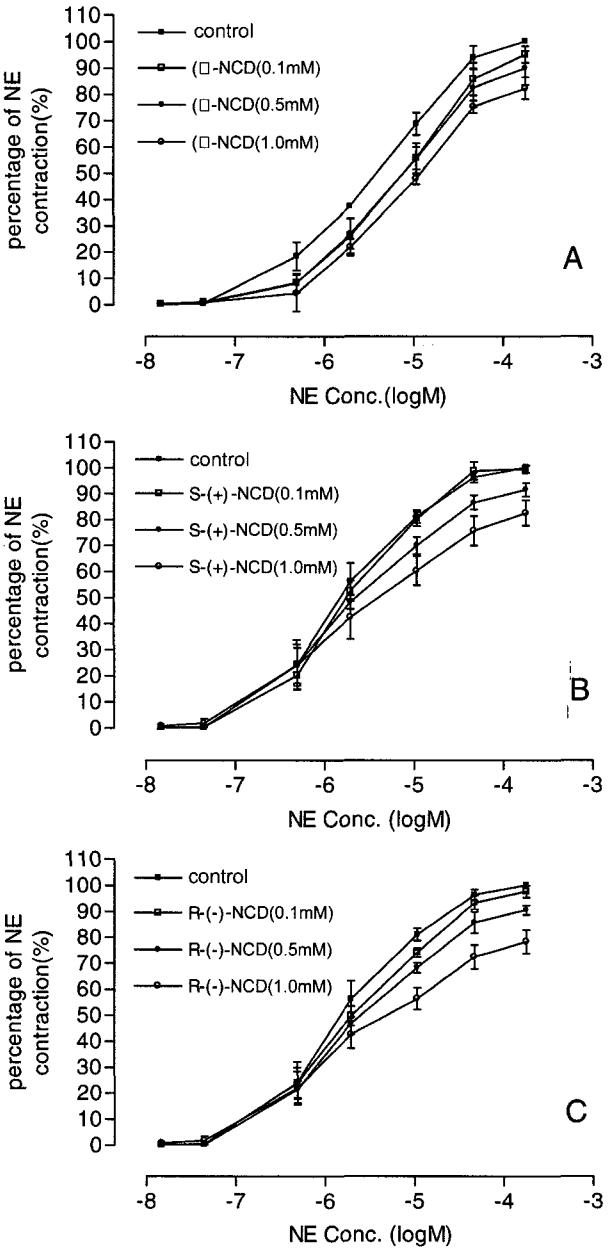

Fig. 3. Inhibitory effect of (±)-NCD(A), *S*-(+)-NCD(B) and *R*-(-)-NCD(C) on the vasoconstriction evoked by NE in aortic strips of rabbit. Values are means±SEM (n=8).

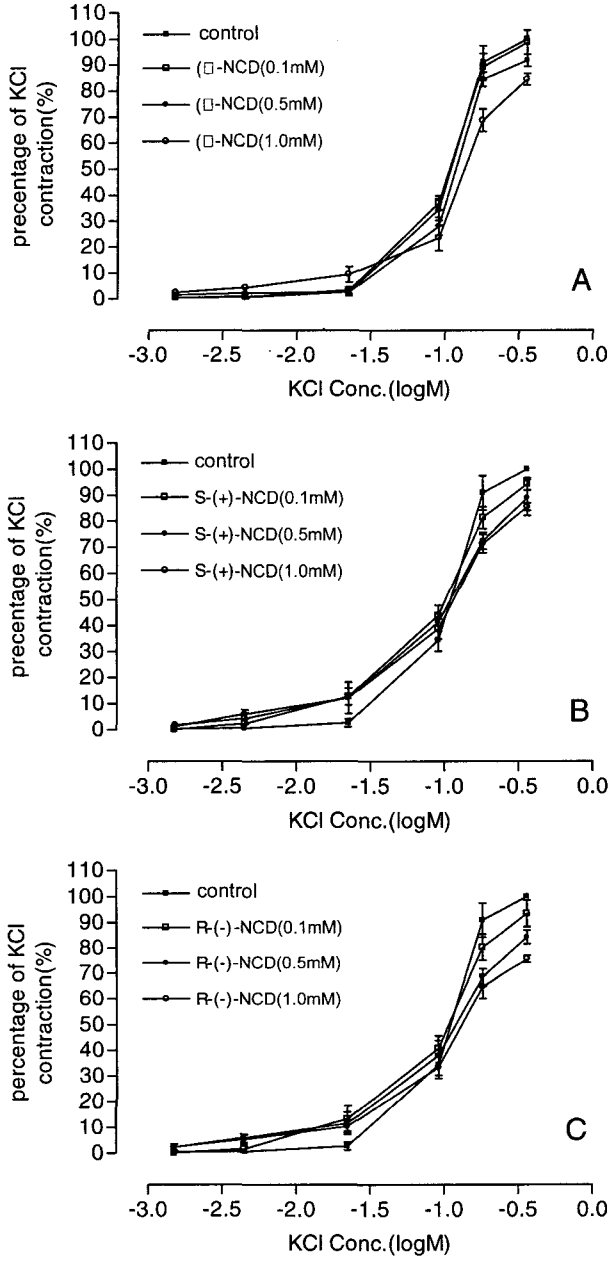

Fig. 4. Inhibitory effect of (±)-NCD(A), *S*-(+)-NCD(B) and *R*-(-)-NCD(C) on the vasoconstriction evoked by KCl in aortic strips of rabbit. Values are means±SEM (n=8).

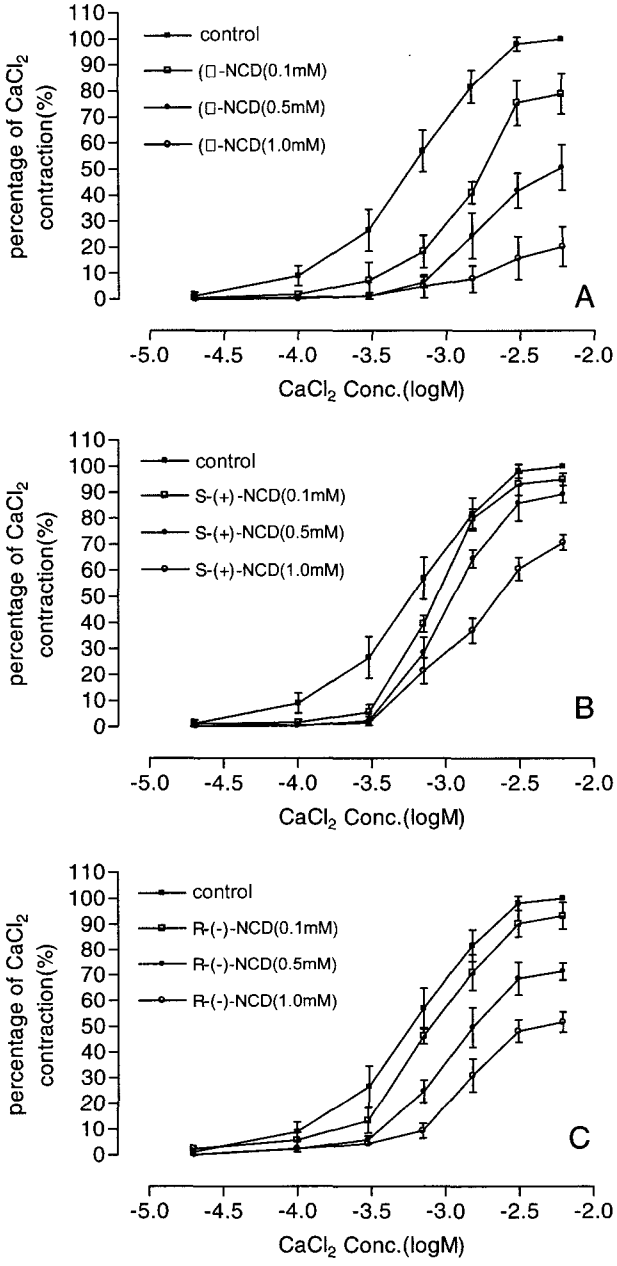

Fig. 5. Inhibitory effect of (±)-NCD(A), *S*-(+)-NCD(B) and *R*-(-)-NCD(C) on the vasoconstriction evoked by $CaCl_2$ in aortic strips of rabbit. Values are means±SEM (n=8).

(±)-NCD, *S*-(+)-NCD and *R*-(-)-NCD could shift the doseresponse curves to right in a nonparallel manner (Fig. 3 for NE, Fig. 4 for KCl and Fig. 5 for $CaCl_2$), and the E_{max} were decreased in a concentration-depended manner, respectively. The E_{max} and the pD_2' values are showen in Table I, Table II and Table III for NE, KCl and $CaCl_2$ respectively.

The pD_2' values of (±)-NCD, *S*-NCD and *R*-NCD were calculated (Kanamori *et al.*, 1981) and listed in Table I, Table II and Table III. Using the *q* test, the results showed that inhibition of *R*-NCD on NE, KCl and $CaCl_2$ was significantly stronger than that of *S*-NCD and (±)-NCD ($p<0.01$). There was no obvious difference between (±)-NCD and *S*-NCD on the three models. *R*-NCD was able to more efficiently inhibit the NE, high K^+ and high Ca^{2+} induced contractile response of rabbit aortic strips.

DISCUSSION

The suitable way to evaluate the calcium antagonists is

Table I. Effect of (±)-NCD, *S*-(+)-NCD, and *R*-(-)-NCD on NE-induced vasoconstriction in strips of rabbit aortic. Values are shown as means±SD (n=8)

Conc. ($mmol \cdot L^{-1}$)		E_{max} (%)	pD_2	pD_2'
control		100	5.78±0.08	
(±)-NCD	0.1	95±3		4.39±0.1
	0.5	85±6		
	1.0	82±4		
S-(+)-NCD	0.1	99±1		3.97±0.1
	0.5	91±3		
	1.0	82±4		
R-(-)-NCD	0.1	97±2		4.55±0.3
	0.5	90±2		
	1.0	78±4		

Table II. Effect of (±)-NCD, *S*-(+)-NCD, and *R*-(-)-NCD on KCl-induced vasoconstriction in aortic strips of rabbit. Values are shown as means±SD (n=8)

Conc. ($mmol \cdot L^{-1}$)		E_{max} (%)	pD_2	pD_2'
control		100	1.24±0.05	
(±)-NCD	0.1	98±5		4.88±0.1
	0.5	92±2		
	1.0	84±2		
S-(+)-NCD	0.1	94±2		4.76±0.1
	0.5	90±7		
	1.0	86±2		
R-(-)-NCD	0.1	93±5		5.01±0.3
	0.5	84±3		
	1.0	76±1		

Table III. Effect of (±)-NCD, *S*-(+)-NCD, and *R*-(-)-NCD on $CaCl_2$-induced vasoconstriction in aortic strips of rabbit. Values are shown as means±SD(n=8)

Conc. ($mmol \cdot L^{-1}$)		E_{max} (%)	pD_2	pD_2'
control		100	3.33±0.10	
(±)-NCD	0.1	79±8		6.17±0.3
	0.5	51±9		
	1.0	20±8		
S-(+)-NCD	0.1	95±2		6.03±0.1
	0.5	89±3		
	1.0	71±3		
R-(-)-NCD	0.1	93±5		6.48±0.2
	0.5	71±3		
	1.0	52±4		

to use the high K^+ concentration to open the PDC combined with the receptor activator to active the ROC method (Hof RP, 1983). In the present study, we found that (±)-NCD and its eanatiomers have the ability to antagonize the contraction of aortic strips induced by NE, KCl and $CaCl_2$ *in vitro*. Thus, (±)-NCD and its eanatiomers reduced the vascular tone of isolated aortic rings that was controlled by two different receptors in a similar manner. Therefore, (±)-NCD and its eanatiomers elicited a direct effect on the vascular smooth muscle. Thus, we did not repeat the experiments regarding the effect of (±)-NCD on calcium influx.

The results showed that *R*-(-)-NCD's effect is characterized by a tissue selectivity that is different from that of (±)-NCD and *S*-(+)-NCD, which have a similar affinity for vascular Ca^{2+} channels. The pD_2' value for the effect of *R*-(-)-NCD on the contractile response to $CaCl_2$ is about 6.5, and this is obviously higher than those of (±)-NCD ($P<0.01$). Furthermore, the effects of *R*-(-)-NCD on contractile response to NE and $CaCl_2$ show that *R*-(-)-NCD can combine with the stereoselective receptor on aortic smooth muscle. It can inhibit not only the activity of NE on the ROC, which decreases in intercellular Ca^{2+} release, but it also that of NE on the PDC activation to block the intracellular Ca^{2+} influx into cell.

In conclusion, *R*-NCD's inhibition was significantly stronger than that of *S*-NCD and (±)-NCD. The results reflect that the interaction between *R*-NCD and the L-calcium channel receptor is more stereoselective and stronger than that between the other enantiomers. *R*-NCD was able to produce a strong pharmacological effect. For the safety and validity of nicardipine used in clinical settings, it is very reasonable to develop and utilize only *R*-NCD.

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