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Characteristics of tetrahydroprotoberberines on dopamine D_1 and D_2 receptors in calf striatum[1]

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KEY WORDS tetrahydroprotoberberines; Sch-23390; spiperone; guanosine triphosphate; corpus striatum; dopamine D_1 receptors; dopamine D_2 receptors; radioligand assay; stepholidine; 12-chloroscoulerine

AIM: To study the characteristics of tetrahydroprotoberberines (THPB) on dopamine D_1 and D_2 receptors and elucidate their structure-activity relationship. **METHODS:** Radioligand assay *in vitro* with a two-site model program analysis. **RESULTS:** Four THPB with two hydroxyl groups on C_2 and C_9 or C_2 and C_{10} exhibited R_H and R_L two binding sites to D_1 receptors and guanosine triphosphate regulated the R_H binding site of SPD and THPB-132A in competition assay, while eleven THPB including nonhydroxy-THPB, monohydroxy-THPB, and THPB with two hydroxyl groups attaching to C_3 and C_{10} showed one binding site to D_1 receptors under the same conditions. However, the tested eleven THPB all manifested one binding site to D_2 receptors in competition assay, and the 2-hydroxy-THPB had the most potent affinity for D_2 receptors. **CONCLUSION:** Dihydroxy-THPB with two hydroxyl groups attaching to C_2 and C_9 or C_2 and C_{10} possess the intrinsic activity of agonist to D_1 receptors, while the other THPB do not. The tested eleven THPB all are the antagonists of D_2 receptors.

Tetrahydroprotoberberines (THPB) are the serial alkaloids isolated from Chinese herb *Stephania* or synthesized compounds. THPB analogs share the common structure of isoquinoline ring and methoxyl groups or hydroxyl groups at position C_2, C_3, C_9, and C_{10}. THPB are novel active compounds on the brain DA receptors. THPB possess the affinities for D_1 and D_2 receptors, with a preference for D_1 receptors[1-2]. (−)-Stepholidine (SPD), a leading compound of THPB, possesses dual actions, agonistic to D_1 and antagonistic to D_2 receptors[3-5].

DA receptors couple to adenyl cyclase (AC) through G protein. Sch-23390, a selective D_1 receptor antagonist, completely blocks the DA-stimulated AC activity. [^{3}H]Sch-23390 thus labels a homogeneous population of D_1 receptors. An antagonist competition for [^{3}H]Sch-23390 binding displays a monophasic curve. In contrast, when an agonist competes for [^{3}H]Sch-23390 binding, the resultant competition curve is more shallow. The agonist competition consistently models best to a two-site curve composed of a high affinity site (R_H) and a low affinity site (R_L)[6]. After addition of guanosine triphosphate (GTP), the R_H of agonist binding site is converted to the R_L[7]. On the other hand, the antagonist competition curve for [^{3}H]spiperone (a selective D_2 receptor antagonist) binding fits best to a single-site model, while the agonist/[^{3}H]spiperone curve fits best to a two-site model. In the presence of GTP, the agonist curve shifts to the right and fits best to a single-site model[8].

The present study aimed to investigate the action of THPB to D_1/D_2 receptors and the structure-activity relationship between THPB and DA receptors.

MATERIALS AND METHODS

Chemicals THPB are listed in Tab 1. SPD was isolated from the Chinese herb *Stephania intermedia* Lo. Other THPB were synthesized by Shanghai Institute of Materia Medica. They were dissolved in a small amount of *N*,*N*-dimethyl formamide (DMF) and then diluted with

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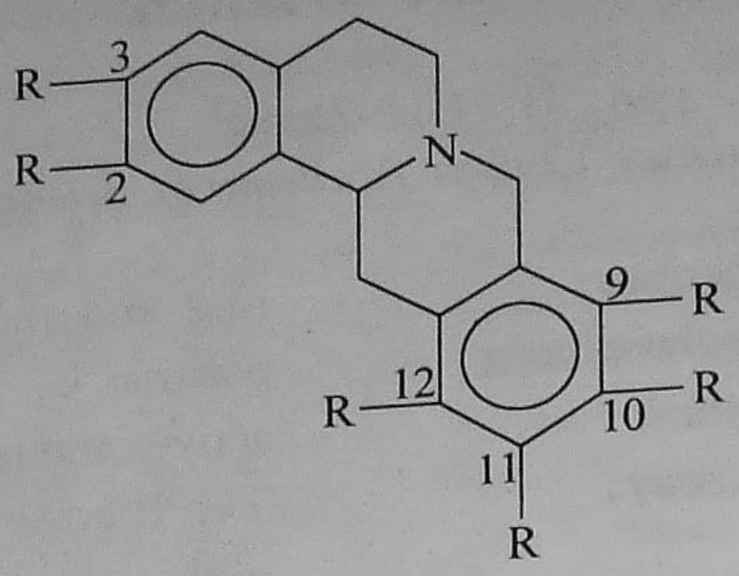
Tab 1. Chemical structures of tetrahydroprotoberberines (THPB).



Compound		R 2	3	9	10	11	12
Nonhydroxy-THPB	THB	O−CH_2−O		OCH_3	OCH_3	H	H
	THP	OCH_3	OCH_3	OCH_3	OCH_3	H	H
Monohydroxy-THPB	THPB-126A	OCH_3	OCH_3	Cl	OH	H	H
	THPB-126	OCH_3	OCH_3	H	OH	Cl	H
	THPB-104	OCH_3	OCH_3	H	OH	OCH_3	Cl
	THPB-143	OH	OCH_3	H	H	H	H
	THPB-10	OCH_3	OCH_3	OH	OCH_3	H	Cl
Dihydroxy-THPB	THPB-161	OCH_3	OH	H	OH	Cl	H
	THBP-161A	OCH_3	OH	Cl	OH	H	H
	THPB-1	OH	OH	OCH_3	OCH_3	H	H
	THPB-107	OH	OCH_3	H	H	OCH_3	OH
	THBP-14	OH	OCH_3	H	OH	OCH_3	H
	THBP-132	OH	OCH_3	H	OH	Cl	H
	THBP-132A	OH	OCH_3	Cl	OH	H	H
	SPD	OH	OCH_3	OCH_3	OH	H	H
	CSL	OH	OCH_3	OH	OCH_3	H	Cl
	THBP-57	OH	OCH_3	H	OH	OCH_3	Cl

reaction medium. [^{3}H]Sch-23390 (2.92 PBq·mol^{-1}) and [^{3}H] spiperone (3.77 PBq·mol^{-1}) were purchased from Amersham. All other reagents were of AR: Sch-23390 and butaclamol (RBI, USA); apomorphine (Apo) and Na_2GTP (Sigma, USA). Other reagents were of AR.

Membrane preparation Male calf (Shanghai Milk Co) was killed and brains were rapidly excised. The striatum was homogenized in ice-cold Tris-HCl buffer 50 mmol·L^{-1} (pH 7.4). The homogenate was spun at 1500×*g* at 4 ℃ for 10 min. The supernatant was spun at 20 000×*g* at 4 ℃ for 20 min. The pellet was rinsed once with the buffer, and was suspended in the D_1 or D_2 assay buffer. The D_1 assay buffer was composed of Tris-HCl 50 mmol·L^{-1}(pH 7.4), $MgSO_4$ 5 mmol·L^{-1}, edetic acid 0.5 mmol·L^{-1} and 0.02 % ascorbic acid. The D_2 assay buffer was composed of Tris-HCl 50 mmol·L^{-1} (pH 7.4), edetic acid 0.5 mmol·L^{-1}, KCl 5 mmol·L^{-1}, NaCl 100 mmol·L^{-1}, $CaCl_2$ 2 mmol·L^{-1}, $MgCl_2$ 4 mmol·L^{-1} and 0.01 % ascorbic acid. Protein content was measured[9].

Receptor binding assay Incubation was initiated by adding tissue (0.4 mg protein/tube) to duplicate tubes containing labeled ligand and unlabeled competitor (with or without GTP) to yield a 1.0 mL final assay volume. [^{3}H]Sch-23390 and [^{3}H]spiperone were used as radioligands to label D_1 and D_2 receptors, respectively. Nonspecific binding was defined by Sch-23390 100 nmol·L^{-1} for D_1 or by butaclamol 1 μmol·L^{-1} for D_2 receptors. After incubation at 37 ℃ for 30 min, the reaction was stopped by ice-cold Tris-HCl buffer. Rapid filtration was done through Whatman glass fiber filters (GF/C) with reduced pressure. The filters

were rinsed with Tris-HCl buffer thrice, dried at 80 ℃, and counted by LKB scintillation spectroscopy (efficiency =62 %).

Data analysis The weighted nonlinear curve fitting program GraphPAD InplotVS3 was used for the analysis of saturation and competition experiments. Competition curves were initially analyzed with a single-site model and then with a two-site model. The results of the two-site analysis were compared with the single-site analysis by F test. The two-site model was retained if it fitted the data better than the single-site model ($P<0.05$).

RESULTS

Saturation analysis of [^{3}H]Sch-23390 binding The binding of [^{3}H]Sch-23390 to D_1 receptors in calf striatum homogenates showed a saturable curve. Nonspecific binding increased linearly over the entire concentration range (0.125 – 16 nmol · L^{-1}) and never exceeded 10 % of total binding. K_d was 1.65 ± 0.21 nmol · L^{-1} and B_{max} was 908 ± 51 pmol/g protein ($n=3$). The Hill coefficient was 0.91, indicating a single binding site of radioligand to D_1 receptors.

THPB competition for [^{3}H]Sch-23390 binding The abilities of Sch-23390, Apo and fifteen THPB to compete for [^{3}H]Sch-23390 (final concentration 0.25 nmol · L^{-1}) binding in calf striatal homogenates were measured.

The competition curve of Sch-23390 for [^{3}H]Sch-23390 binding was best fit to a single, homogeneous population of binding sites with the K_i value of 1.7 ± 2.9 nmol · L^{-1} ($n=3$). Apo/[^{3}H]Sch-23390 curve modeled best to a heterogeneous two-site fit with the R_H of 38 % ±5 %, the K_i values for Apo at high (K_H) and low (K_L) affinity sites were 2.7 ± 0.4 and 378 ± 62 nmol · L^{-1}, respectively ($n=3$). Addition of GTP 0.3 mmol · L^{-1} converted the R_H to the R_L, thus the Apo/[^{3}H]Sch-23390 curve modeled best to a single-site fit with the K_i values of 155 ± 39 nmol · L^{-1} ($n=3$).

Among the fifteen THPB, the competition curves of four compounds (SPD, THPB-132A, CSL, and THPB-57) for [^{3}H]Sch-23390 binding modeled best to a two-site fit. In the presence of GTP 0.3 mmol · L^{-1}, the curves of SPD and THPB-132A (Fig 1) modeled best to a single-site fit. However, no effect of GTP on the R_H of THPB-57 and (±) 12-chloroscoulerine (CSL) binding was observed, their curves modeled best to a two-site fit still (Tab 2).

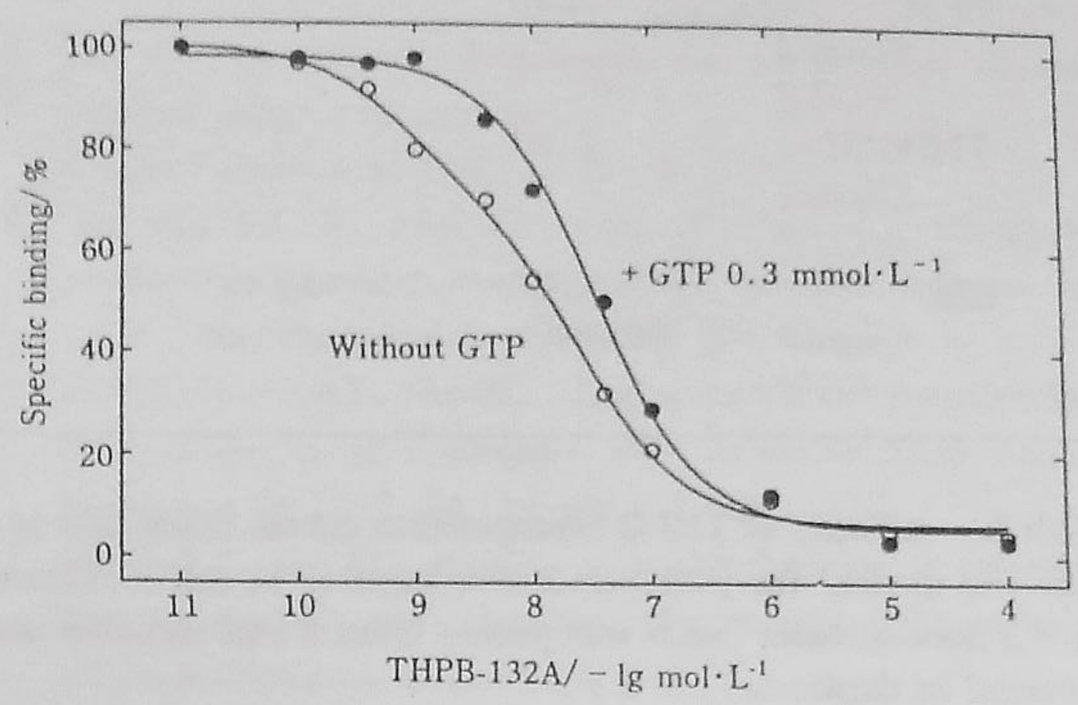


Fig 1. Computer-fitted curves for THPB-132A inhibition of [^{3}H]Sch-23390 0.8 nmol·L^{-1} binding to calf striatum in the absence (○) and presence (●) of GTP 0.3 mmol·L^{-1}.

GTP 0.6 or 0.9 mmol · L^{-1} did not alter the binding property of THPB-57 and CSL either (unpublished data). In contrast to the four THPB, the curves of other eleven THPB modeled best to a single-site fit (Tab 3). As to the values of K_i, THPB with one or two hydroxyl groups at C_2, C_9, or C_{10}, but not C_3, had higher affinity for D_1 receptors than nonhydroxy-THPB. THPB with chlorine atom at C_{12} had high affinity, while THPB with chlorine atom or methoxyl group at C_{11} had low affinity.

Saturation analysis of [^{3}H]spiperone binding The binding of [^{3}H]spiperone to D_2 receptor in calf striatum homogenates showed a saturable curve. Nonspecific binding increased linearly over the entire concentration range (0.125 – 16 nmol · L^{-1}) and never exceeded 10 % of total binding. K_d was 0.48 ± 0.05 nmol · L^{-1} and the B_{max} was 126 ± 23 pmol/g protein. The Hill coefficient was 0.99, indicating a single binding site of radioligand to D_2 receptors.

THPB competition for [^{3}H]spiperone binding The competitive curve of Apo for [^{3}H] spiperone (final concentration 0.25 nmol · L^{-1}) binding fitted

Tab 2. Parameters for THPB (competition curves fitted best to a two-site model) inhibition of [^{3}H]Sch-23390 binding to calf striatum in the absence and presence of GTP 0.3 mmol·L^{-1}. $n = 3$ homogenates (each was pooled from 4 calf striatum and assayed in duplicate), $\bar{x} \pm s$.

Group	OH position	R on C_{12}	K_i/mmol·L^{-1}	K_L/nmol·L^{-1}	K_H/nmol·L^{-1}	R_H/%
THPB-132A	2,10	–	–	19.7±0.3	0.66±0.03	32±4
Control			21.4±0.3	–	–	–
+GTP			–	119±49	3.1±1.8	41±4
SPD	2,10	–	–	–	–	–
Control			280±49			
+GTP						
THPB-57	2,10	Cl	–	14.1±0.7	0.66±0.05	50±9
Control			–	16.9±0.6	0.18±0.01	50±7
+GTP						
CSL	2,9	Cl	–	80±11	0.76±0.02	70±5
Control			–	222±15	7.2±0.6	68±6
+GTP						

Tab 3. Affinity of THPB (competition curves fitted best to a one-site model) for [^{3}H]Sch-23390 binding to calf striatum. $n = 3$ homogenates (each was pooled from 4 calf striatum and assayed in duplicate), $\bar{x} \pm s$.

Compound	OH position	R at C_{11}	K_i/nmol·L^{-1}
THB	–	–	467±39
THP	–	–	754±56
THPB-126A	10	–	83±11
THPB-126	10	Cl	433±22
THPB-104	10	OCH_3	230±10
THPB-143	9	–	150±16
THPB-161	3,10	Cl	>10 000
THPB-161A	3,10	–	>1 000
THPB-107	2,12	OCH_3	364±76
THPB-14	2,10	OCH_3	66±6
THPB-132	2,10	Cl	138±46

Tab 4. Affinity of THPB for [^{3}H]spiperone binding to calf striatum. $n = 3$ homogenates (each was pooled from 4 calf striatum and assayed in duplicate), $\bar{x} \pm s$.

Compound	OH position	K_i/μmol·L^{-1}
THB	–	101±16
THP	–	122±12
THPB-126	10	117±21
THBP-104	10	168±28
THPB-10	9	73±9
THPB-143	2	2.4±0.6
THPB-1	2,3	18±5
THBP-132A	2,10	9.6±0.7
SPD	2,10	7.2±0.8
THPB-57	2,10	7.5±1.3
CSL	2,10	5.9±0.4

best to a two-site model with a high affinity site ($K_H = 2.9 \pm 0.7$ nmol·L^{-1}, $n = 3$) and a low affinity site ($K_L = 722 \pm 142$ nmol·L^{-1}, $n = 3$). After addition of GTP, the Apo binding curve modeled best to a single site fit with K_i value of 228 ±24 nmol·L^{-1} ($n = 3$). In contrast to Apo, all tested THPB modeled best to a single-site fit.

As to the values of K_i, THPB with single hydroxyl group at C_2 were more potent than those with hydroxyl group at C_9 or C_{10}. When the hydroxyl group was substituted by methoxyl or methylenedioxyl group, the affinity of THPB for D_2 receptors were decreased (Tab 4).

DISCUSSION

In the present experiments, the competition curves of SPD, THPB-132A, and DA receptor agonist Apo were best fit to a two-site model, and the addition of GTP converted the curves to fit best to a single-site model. Under the same conditions, the curves of other eleven THPB and D_1 receptor antagonist Sch-23390 were best fit to a single-site model. These results suggested that SPD and THPB-132A possess the intrinsic activity of agonist to D_1 receptors, while other eleven THPB did not. The previous biochemical and behavioral observations have demonstrated that SPD exhibited the action of D_1 agonist, while THP and THB did not[4-5]. The further study about THPB analogs also found that THPB-132A induced the contralateral rotation in 6-OHDA lesioned rats, while other THPB did not (to be published). These results obtained in functional studies are in

line with the present results obtained in radioligand assay.

The behavioral studies had demonstrated that CSL[10] and THPB-57 (to be published) had the D_1 agonistic action as SPD did. Surprisingly, the competition curves of CSL and THPB-57 for [^{3}H]Sch-23390 binding fitted best to a two-site model, but the R_H could not be diminished by GTP. According to the theory of tenary complex model[6], the binding of GTP to G protein promotes instability of an agonist-receptor-G protein (L-R-G) ternary complex and a concomitant reduction or abolition of the R_H site. Thus, it suggested that CSL and THPB-57 possess the intrinsic activity of agonist to D_1 receptors, but GTP could not regulate the R_H site owing to the interference of the dissociation of L-R-G complex.

Based on the structure-activity relationship, the present results suggested that dihydroxy-THPB possess the intrinsic activity of agonist to D_1 receptors, while nonhydroxy-THPB and monohydroxy-THPB did not. Furthermore, the positions of two hydroxyl groups attaching to THPB is an important factor on D_1 receptor binding. THPB with two hydroxyl groups on C_2 and C_9 or C_2 and C_{10}, but not C_3 and C_{10} possessed the intrinsic activity of agonist to D_1 receptors. Nevertheless, the dihydroxy-THPB with the substitution of chlorine atom or methoxyl group for hydrogen atom at C_{11} of THPB would abolish or reduce the D_1 intrinsic activity, such as THPB-14, THPB-132. However, the chlorine atom at C_{12} of dihydroxy-THPB would interfere with the action of GTP on the THPB binding to D_1 receptors, such as CSL and THPB-57.

As to D_2 receptors, our results demonstrated that Apo/[^{3}H]spiperone curve fitted best to a two-site model and could be regulated by GTP. In contrast to the D_2 agonist Apo, the curves of eleven THPB fitted best to a single-site model, suggesting their action of D_2 antagonist. This is consistant with the electrophysiological and biochemical observations[11-13]. Among eleven THPB, the 2-hydroxy-THPB compounds had the most potent affinity to D_2 receptors, which corresponded with the previous results[11].

In conclusion, two hydroxyl groups located on C_2 and C_9 or C_2 and C_{10} of THPB is the structural element responsible for the D_1 instrinsic activity of THPB.

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四氢原小檗碱类对小牛纹状体 D_1 和 D_2 多巴胺受体结合的特性[1]

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关键词 四氢原小檗碱类；Sch-23390；螺哌隆；鸟苷三磷酸；纹状体；多巴胺 D_1 受体；多巴胺 D_2 受体；放射配位体测定；千金藤立定；12-氯代斯阔任

目的：研究四氢原小檗碱类(THPB)对脑内多巴胺受体 D_1 和 D_2 亚型的结合特性，并阐明它们之间的构效关系． 方法：放射配位体测定结合双位点模型分析． 结果：4 个 THPB 与 D_1 受体以 R_H 和 R_L 双位点结合，它们在 C_2 和 C_9 或 C_2 和 C_{10} 位有两个羟基，另外 11 个 THPB 与 D_1 受体以单位点结合． 对于 D_2 受体，11 个被检测的化合物均以单位点结合，其中，在 C_2 位有羟基的 THPB 亲和力最强． 结论：在 C_2 和 C_9 或 C_2 和 C_{10} 位有双羟基的 THPB 具有 D_1 受体激动剂的内在活性，其它 THPB 则无此活性． 11 个 THPB 均为 D_2 受体拮抗剂．

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Carbamazepine facilitates effects of GABA on rat hippocampus slices

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KEY WORDS carbamazepine; GABA; baclofen; hippocampus; evokedpotentials

AIM: To study the influence of carbamazepine (Car) on GABA effect in hippocampus. METHODS: Evoked potentials were recorded on pyramidal cells in CA1 after stimulation (0.5 Hz, 50 μs) to Schaffer collaterals in rat hippocampal slices (350 μm). RESULTS: Car 0.1 and 0.2 mmol·L^{-1} did not affect field potentials, whereas Car 0.2 mmol·L^{-1} plus GABA (0.1－1 mmol·L^{-1}) gave rise to a stronger inhibition on field potentials than that of GABA alone. Bicuculline did not reverse Car facilitation on GABA inhibition on field potentials. (－)-Baclofen was more effective in inhibiting field potentials than GABA. Car 0.2 mmol·L^{-1} plus (－)-baclofen (1－5 μmol·L^{-1}) brought an inhibition stronger than that of (－)-baclofen alone. CONCLUSION: Car facilitates the effects of GABA on pyramidal cells in hippocampal CA1 region, probably related to $GABA_B$ receptors.

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The mechanism of the anticonvulsant carbamazepine (Car) is not clear[1,2]. There were some evidences indicating the action of Car is related to GABA system. For instance, picrotoxin, a GABA-regulated chloride ion channel blocker, could work against the taming effect of Car on footshock-induced fighting in mice[3]. Chronic administration of Car increased the GABA concentration in some brain regions of the rat[4,5]. Moreover, alteration of GABA receptors in rat brain was seen after chronic treatment with Car, and the density of $GABA_B$ receptors was enhanced in the hippocampus[6]. The present work was undertaken to examine the interactions of Car with GABA in rat hippocampus *in vitro*.

MATERIALS AND METHODS

Sprague-Dawley ♂ rats weighing 160－200 g were decapitated and the brains were placed in ice-cold Krebs-Ringer solution (NaCl 124, KCl 5, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.6, and glucose 10 mmol·L^{-1}) gassed with 95 % O_2 + 5 % CO_2. Parasagittal slices containing hippocampus (350 μm) were preincubated in Krebs-Ringer solution at 35 ℃ for 90－120 min.

Slices were transferred to a chamber for recording field potentials and perfused with Krebs-Ringer solution at 1.3 mL