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Augmentation of dopamine release by (-)-stepholidine from rabbit and rat caudate slices[1]

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AIM: To study the effect of (-)-stepholidine (SPD) on dopamine (DA) release evoked by electric stimulation on slices of rabbit caudate nucleus. **METHODS**: Slices of rabbit caudate nucleus were preincubated with [^{3}H]DA and then perfused and stimulated electrically. **RESULTS**: Quinpirole (Qui), a selective DA D_2 receptor agonist, reduced [^{3}H]DA overflow elicited by 24 mA electric stimulation (IC_{50} = 0.12, 95 % confidence limits 0.09 — 0.17 $\mu mol \cdot L^{-1}$). SPD markedly increased the potential of the stimulation-evoked [^{3}H]DA overflow in a dose-dependent manner and reversed Qui (1 $\mu mol \cdot L^{-1}$)-induced attenuation of [^{3}H]DA release. The pA_2 value calculated from the data of [^{3}H]DA overflow for SPD was 7.495. In the experiments with rat caudate nucleus slices, SPD (0.1 $mmol \cdot L^{-1}$) increased the [^{3}H]DA outflow from 3.3 ±0.2 % to 6.5±0.5 % of the tissue [^{3}H]DA content, which was further enhanced by the protein kinase C (PKC) activator phorbol 12,13-dibutyrate (PDB, 1 $\mu mol \cdot L^{-1}$) to 10.1 ±1.0 % of the total [^{3}H]DA in the tissue. **CONCLUSION**: SPD is a presynaptic D_2 autoreceptor antagonist and induces a synergic effect on [^{3}H]DA release process with PKC.

KEY WORDS berbines; phorbol 12, 13-dibutyrate; caudate nucleus; dopamine D_2 receptors; protein kinase C; (-)-stepholidine

(-)-Stepholidine (SPD), belonging to the analogs of tetrahydroprotoberberine (THPB), is a dopamine (DA) receptor antagonist[1]. SPD showed a high affinity to DA receptors in the striatum membrane with preference to D_1 receptors[2], antagonized the stereotypy action induced by apomorphine (Apo)[3], and reversed and/or antagonized Apo-induced inhibition on the firing activity of substantia nigra pars compacta (SNC) DA neurons[4,5]. SPD enhanced the tyrosine hydroxylase activity through the inhibition on negative feedback regulation of presynaptic D_2 receptors *in vivo*, followed by increases of DA biosynthesis and DA release[6-8]. In order to further elucidate the DA release by SPD, the present work aims at the effect of SPD on the release of [^{3}H]DA from rabbit and rat caudate slices.

MATERIALS AND METHODS

Materials (-)-Stepholidine (SPD, mp 161—2 C, $[\alpha]_D$-400, Shanghai Institute of Materia Medica Chinese Academy of Sciences), quinpirole (Qui, Research Biochemicals International) and phorbol-12,13-dibutyrate (PDB, Sigma) were dissolved in dimethylformamide (DMFA) as stock solutions at the concentrations of 1 $mmol \cdot L^{-1}$. The maximal DMFA concentration in the incubation medium was 0.1 % (v/v) and had no effect on [^{3}H]DA release. The composition of incubation medium was: NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.57, disodium editic acid 0.03 $mmol \cdot L^{-1}$ (bubbled with 5 % CO_2 in O_2; pH 7.4). [^{3}H]DA (1300 $PBq \cdot mol^{-1}$) and Insta-gel XF were purchased from Amersham. Soluene 350 was purchased from Pakard.

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Methods Rabbits or rats were decapitated. The head of caudate nucleus was bluntly excised from the capsule. Slices (350 μm) were prepared and stimulated as described previously[4].

Caudate nucleus slices were preincubated in the presence of [^{3}H]DA 0.1 μmol·L^{-1} for 30 min and then perfused with the perfusion medium (37 ℃) 1 mL·min^{-1} by a roller pump. The perfusate was collected for 5 min as one sample. During perfusion the slices were stimulated twice (S_1, S_2) for 2 min each after 60 and 100 min by 24 mA constant current. The drugs tested were added to the perfusion medium 10 min before S_2. At the end of the perfusion, the slices were dissolved in 0.25 mL soluene. [^{3}H]DA contents were determined[5]. SPD-evoked [^{3}H]DA release was carried out in rat striatal slices in which SPD was used instead of electric stimulation.

Calculation and statistics[10] The outflow of [^{3}H]DA was expressed as fractional rate: [^{3}H]DA efflux per 5 min/[^{3}H]DA content in the slice tissue at the beginning of this 5 min period × 100 %. Drug effects were quantified as the ratio (S_2/S_1) of the overflow (expressed as % of [^{3}H]DA in tissue) evoked by the 2 stimulation periods. IC_{50} was calculated for agonist that inhibited the electric stimulation-evoked overflow of [^{3}H]DA. The IC_{50} here was defined as the dose, obtained by interpolation, that reduced the control S_2/S_1 ratio by 50 %, and not as the dose that produced 50 % of the maximal inhibition induced by the drug. The pA_2 value of SPD against the release-inhibiting effect of Qui was calculated according to $pA_2 = \lg(E_{An}/E_{Co} - 1) - \lg[An]$. E_{Co} is the mean dose of Qui that caused 50 % inhibition of the stimulation-evoked overflow of [^{3}H]DA (ie, reduced by 50 % the S_2/S_1 ratio) in the absence of antagonist; E_{An} is the mean dose of Qui that caused 50 % inhibition in the presence of antagonist; [An] is the antagonist dose. The results are given as arithmetic $\bar{x} \pm s$. The significance of the differences between the means of variance was followed by a supplementary two-tailed t test.

RESULTS

Control values of [^{3}H]DA outflow The rabbit caudate slices were labeled with [^{3}H]DA 0.1 μmol·L^{-1} for 30 min, and then perfused with the medium. The fractional rate of [^{3}H]DA outflow during the 5-min period just before the first electric stimulation was 1.03 ± 0.12 per 5 min. Then, the first stimulation was applied to the slices by 24 mA constant current at the 60th min, followed by a long-lasting increase of [^{3}H]DA outflow with a peak value of 3.7 ± 0.4 per 5 min. The second electric stimulation at the 100th min induced a similar second peak of [^{3}H]DA outflow which was 3.4 ± 0.8 per 5 min. The ratio of S_2/S_1 was 0.93 ± 0.19 (Fig 1).

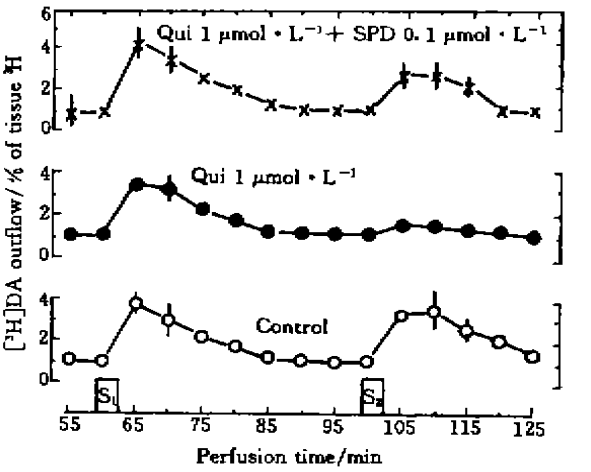

Fig 1. Effect of stepholidine on inhibitory action of quinpirole on tritium outflow from rabbit caudate slices preincubated with [^{3}H] dopamine (n = 6 rabbits). Perfusion medium was used as control.

Effect of D_2 agonist on [^{3}H]DA outflow The value of [^{3}H]DA induced by the first stimulation was similar to that of control experiment in rabbit caudate slices. After that, the selective D_2 receptor agonist Qui (1 μmol·L^{-1}) was added into the medium to perfuse for 10 min. The Qui profoundly reduced the [^{3}H]DA overflow evoked by the second electric stimulation with an S_2/S_1 ratio of 0.20 ± 0.05 (Fig 1).

Qui 0.01 — 1.0 μmol·L^{-1} decreased [^{3}H]DA overflow in a dose-dependent manner (Tab 1) and the IC_{50} was 0.12 μmol·L^{-1} (0.09—0.17 μmol·L^{-1}).

Tab 1. Ratio of S_2-stimulated overflow/S_1-stimulated overflow of [^{3}H] (S_2/S_1) in rabbit caudate slices after (-)-stepholidine and quinpirole. n = rabbits in parentheses. $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

Drug μmol·L^{-1}	Quinpirole	SPD	SPD 0.1 μmol·L^{-1} +Quinpirole
0	1.14± 0.17 (6)	0.79± 0.06 (14)	1.11± 0.01 (4)
0.01	1.11± 0.28 (7)	1.18± 0.12 (5)^b	/
0.1	0.52± 0.07 (7)^c	1.88± 0.13 (8)^c	1.05± 0.07 (5)
1.0	0.20± 0.05 (6)^c	2.53± 0.33 (8)^c	0.59± 0.10 (4)^c
10.0	/	2.65± 0.50 (6)^c	0.15± 0.02 (4)^c

Effects of SPD on [^{3}H]DA outflow In combination with SPD (1 μmol·L^{-1}) in the perfusion medium, the Qui-elicited inhibitory effect on the second electrical stimulation-evoked [^{3}H] DA release was completely reversed (Fig 1). SPD altered the IC_{50} value of Qui from 0.12 to 1.9 μmol·L^{-1} ($P<0.01$, Tab 1, Fig 2). The inhibitory curve of Qui was shifted to the right (Fig 3). It meant that the inhibitory effect of Qui was antagonized by SPD. The pA_2 of SPD was 7.945

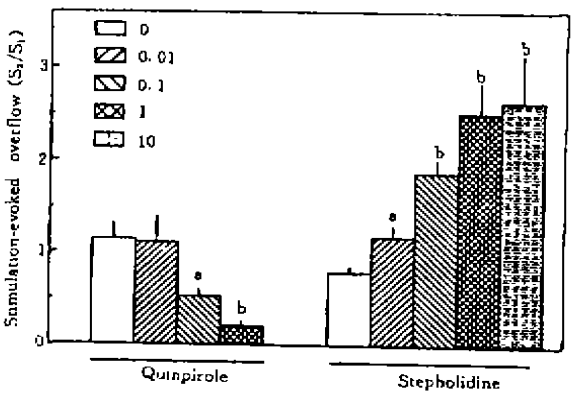

Fig 2. Effect of quinpirole and stepholidine (added 10 min before S_2) on ^{3}H overflow (S_2/S_1) from rabbit caudate slices evoked by electric stimulations. $^aP<0.05$, $^bP<0.01$.

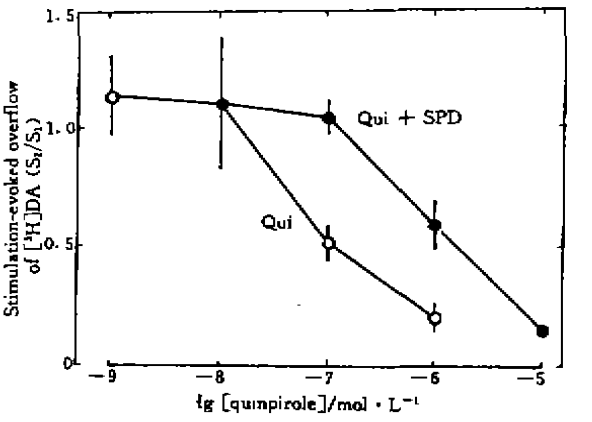

Fig 3. Effects of quinpirlole and stepholidine 1 μmol·L^{-1} (added simultaneously before electric stimulation) on electrically evoked ^{3}H overflow (S_2/S_1).

calculated from the ratios of S_2/S_1.

SPD (10 μmol·L^{-1}) perfused for 10 min profoundly increased the [^{3}H]DA overflow induced by the 2nd stimulation with S_2/S_1 ratio from 0.79±0.06 to 2.6±0.5 (Fig 4). SPD increased stimulation-evoked [^{3}H] DA overflows in a concentration-dependent manner (Tab 1, Fig 2). These results were contrary to that of Qui as above-mentioned.

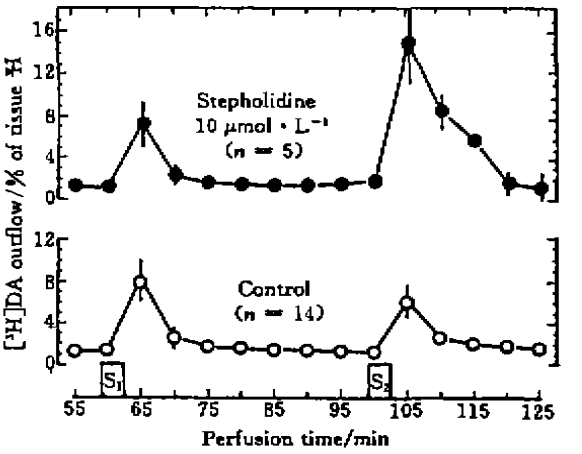

Fig 4. Effect of stepholidine on ^{3}H outflow evoked by electric stimulation.

SPD-evoked [^{3}H] DA release modulated by PDB On the rat slices, after perfusion

with the control medium for 65 min, the SPD added (100 μmol·L^{-1}) distinctly increased the outflow of [^{3}H]DA, which reached the peak value (6.50±0.05/5 min) at the 80th min. Then the outflow decreased to the base line at the 95th min. When the slices were preperfused with PDB (an activator of protein kinase C, 1 μmol·L^{-1}) for 15 min, the [^{3}H]DA outflow was not affected. When the SPD was simultaneously perfused with PDB, a distinct enhancement of the [^{3}H]DA outflow appeared with a peak value of 10.1±1.0, which was a facilitatory effect of PDB in comparison with addition of SPD only (Fig 5). The lower dose of PDB (0.1 μmol·L^{-1}) in the perfusion medium caused the [^{3}H]DA outflow peak values as 13.4±1.0 times against control value.

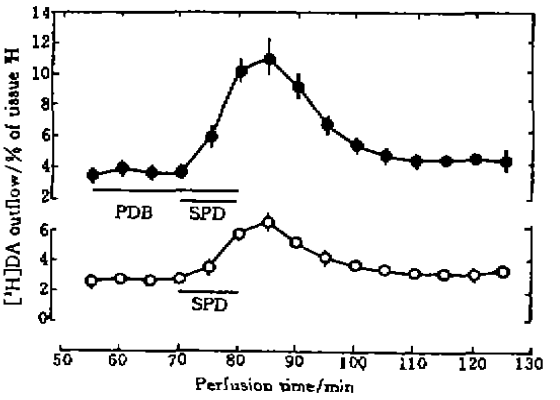

Fig 5. Effect of PDB on SPD-evoked [^{3}H]DA release from rat caudate slices. Lower panel: SPD (100 μmol·L^{-1}) was added for 10 min (n=4 rabbits). Upper panel: the slices were preincubated with PDB (1 μmol·L^{-1}) for 15 min and then perfused together with SPD (1 μmol·L^{-1}) for another 10 min (n=4 rabbits).

DISCUSSION

The previous work[9] has shown that the caudate slice model can be used to study quasi-physiologically release of DA and to test whether a compound is an agonist or antagonist at the DA receptors. Based on the functional and pharmacological profiles, DA receptors have been classified into D_1 and D_2 subtypes. The DA release from presynaptic nervous ending is controlled by D_2 DA autoreceptors contributing to a negative feedback regulation[11]. It has been established that DA D_2 receptor agonists inhibit DA release from dopaminergic nervous ending. Conversely, the D_2 DA receptor antagonists facilitate the DA release.

The results in the present paper showed that the Qui, a selective D_2 dopamine autoreceptor agonist, significantly and dose-dependently inhibited the electrically elicited [^{3}H]DA overflow. SPD, however, elevated the DA release in a dose-dependent manner. These results indicated that SPD had an antagonist effect on DA release via a different mechanism from that of Qui. In general, D_2 DA receptor antagonist can reverse the effect of agonist inhibition on the DA receptors. It has been observed that SPD certainly reversed the inhibitory effect of Qui (1 μmol·L^{-1}) on stimulation-evoked DA release, shifted the response curve of Qui to the right and increased the [^{3}H]DA outflow by stimulation. The pA_2 value calculated from the [^{3}H]DA overflow was 7.945 for SPD. In accordance with the previous results[12], the present work also indicates that SPD is a D_2 DA receptor antagonist.

Moreover, the increase of [^{3}H]DA release induced by SPD during the stimulation was modulated by PDB, an activator of PKC. The activation of PKC in the synaptic region and the following phosphorylation of specific protein might be involved in the mechanism by which PDB promoted NE release[13]. It is suggested that PKC be involved in the mechanism of SPD-evoked DA release.

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左旋千金藤立定加强尾核脑片多巴胺释放

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目的：观察左旋千金藤立定((-)-stepholidine，SPD)对家兔尾核脑片多巴胺(DA)释放的影响. **方法**：以[^{3}H]DA 预孵脑片，测定 DA 放射性. **结果**：选择性 D_2 受体激动剂 LY 171555以剂量依赖方式抑制电诱发的兔尾核脑片[^{3}H]DA 释放. SPD 可翻转 LY 171555对[^{3}H]DA 释放的抑制作用，并以剂量依赖方式加强[^{3}H]DA 释放，在无电刺激条件下，SPD 诱发大鼠尾核[^{3}H]DA 释放被蛋白激酶 C (protein kinase C，PKC)激活剂咐波酯所加强. **结论**：SPD 对突触前 D_2 自身受体是阻滞剂.

关键词 小檗因类；咐波酯；尾状核；多巴胺 D_2受体；蛋白激酶 C；左旋千金藤立定