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Agonistic actions of pergolide on firing activity of dopamine neurons in substantia nigra compacta area

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AIM: To study the potency of pergolide as a D_2 receptor agonist on the firing activity of substantia nigra compacta (SNC) dopamine (DA) neurons compared with that of bromocriptine and to determine whether pergolide has the nature of D_1 receptor agonist *in vivo*. **METHODS**: Extracellular single unit recording techniques. **RESULTS**: Both pergolide and bromocriptine decreased the spontaneously firing rate of "sensitive" and "insensitive" DA cells. In regard of ID_{50} values, pergolide (11.9, 95 % fiducial limits, 5.7—25.1 $\mu g\ kg^{-1}$) was more potent than bromocriptine (7.8, 95 % fiducial limits, 3.3—18.5 $mg\ kg^{-1}$). The discharge inhibition of pergolide was attenuated following the injection of selective D_2 receptor antagonist spiperone 0.25 $mg\ kg^{-1}$ or selective D_1 receptor antagonist Sch-23390 1 — 2 $mg\ kg^{-1}$. However, the inhibition caused by bromocriptine was not always attenuated by spiperone. **CONCLUSION**: Pergolide is 650 times more potent than bromocriptine at D_2 receptors, and possesses D_1 receptor agonist characteristics *in vivo*.

KEY WORDS pergolide; bromocriptine; spiperone; substantia nigra; dopamine receptors; electrophysiology

Dopamine (DA) receptors are divided into 2 subtypes: D_1 coupled with adenylate cyclase (AC) activation positively, and D_2 negatively linked to AC or unrelated to its inhibition[1]. One important location of D_2 receptors in central nervous system (CNS) is on presynaptic soma/dendrites of DA neuron in substantia nigra compacta (SNC). This subpopulation of D_2 receptors is termed autoreceptors as well, with which DA receptor agonists preferentially interact to inhibit the firing activity of DA cells, while the D_2 receptor antagonists reverse or block the suppression[2,3]. This criterion is applied to evaluate the effect of a drug on DA receptors qualitatively and quantitatively. In addition, the major abnormality in parkinsonism is due to the loss of DA neurons in SNC, and administration of D_2 receptor agonists is an effective remedy. Also, D_2 receptors exist on the mammotrophic cells. D_2 receptor agonists thus act on this site to inhibit the secretion of prolactin[4]. Therefore D_2 receptor agonists are used in the treatment of both Parkinson's disease and such endocrine disorders as hyperprolactinemia, acromegaly, and certain pituitary tumors.

Bromocriptine, a D_2 receptor agonist, is currently used as a routine drug in clinic. And pergolide is a new one of high potency. Pergolide is undoubtedly a D_2 receptor agonist both *in vivo* and *in vitro* and a D_1 receptor agonist *in vitro*[5]. However, whether it activates D_1 receptors *in vivo* remains unclear and the reports on its electrophysiology on D_1 receptors *in vivo* have not been found to date. Hence, we compared the potencies of pergolide and bromocriptine on D_2 receptors and

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tested if pergolide influenced D_1 subtype *in vivo*.

MATERIALS AND METHODS

Drugs and reagents Gallamine triethiodide (Sigma); pontamine sky blue (Merck); lidocaine-HCl (Haipu Pharmaceutical Factory, Shanghai); spiperone (Sigma); pergolide mesylate (Tianjin Institute of Materia Medica) was dissolved in normal saline adjusted by HCl 0.1 mol L^{-1} to pH 5. Bromocriptine (Sigma) was dissolved in ethanol and then equal amount of tartrate was added. This solution was diluted with normal saline until the final concentration of ethanol was 5 %. (Vehicle contains the same ingredients).

Rats and surgery Sprague-Dawley rats (♂, n= 32) weighing 254±s 28 g (Shanghai Experimental Animal Center, Shanghai) were used in accordance with the "Guiding Principles in Care and Use of Animals." Preparative surgery was done under ether anesthesia and a tail vein was cannulated for iv injection of drugs. All incision sites and pressure points were thoroughly infiltrated with lidocaine. After surgery, rats were paralyzed with gallamine triethiodide (16 mg kg^{-1}). Body temperature was maintained at 36 — 38 ℃ with an electric heating pad. ECG was monitored.

Single unit recording techniques Extracellular, single unit recordings were performed in paralyzed rats[6]. A small burr hole, 2.2 mm lateral to midline and 3.2 mm anterior to the lambdoid suture[7], was drilled through the skull for recording in SNC. Electric signals picked up by the glass microelectrode (filled with NaCl 2 mol L^{-1} containing 1 % pontamine sky blue, 3—9 MΩ measured *in vitro*) were amplified and led into a window discriminator or displayed on an oscilloscope. Firing rate was counted by a computer.

The identification of neurons as putative SNC DA cells was based on the well-established indices[6,8]. After 5-min period of stable baseline was recorded, drugs were given iv 2 min apart. Each dose was equal to the previous cumulative dose of the same drug. Only one cell was monitored per rat.

Histological examination At the end of experiment, the terminal recording site was marked by passing 15 μA negative current through the recording barrel for 20 min. The rats were then perfused with normal saline containing heparin followed by 10 % buffered formaline. Serial coronal sections were cut (50 μm), then stained with cresyl violet, and counterstained with neutral red. The dye spot was observed under light microscope and served as a reference point.

Statistical procedure The doses of drugs produced a 50 % reduction in activity (ID_{50}) were determined by logit method. Other data were expressed as $\bar{x}\pm s$ and calculated with two-tail t test.

RESULTS

Agonistic effects of pergolide predominantly on D_2 receptors In paralyzed rats, 11 out of 14 DA neurons were sensitive to pergolide (ID_{50}<20 μg kg^{-1}). When a 5 μg kg^{-1} dose was given, firing rate was profoundly depressed to 75.9 % *vs* baseline. More apparent inhibition was observed after larger doses. ID_{50} for pergolide was 11.9 μg kg^{-1} (95 % fiducial limits, 5.7—25.1 μg kg^{-1}). In vehicle group (n = 6), no significant response emerged (Fig 1).

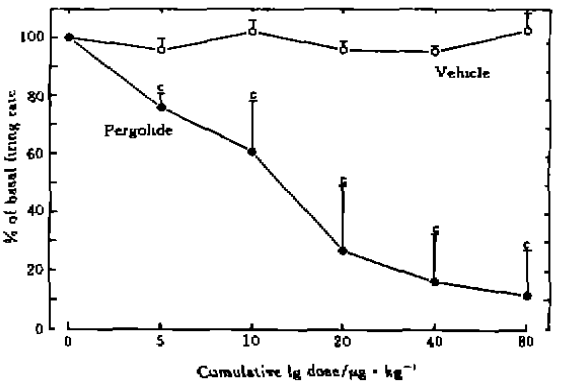

Fig 1. Inhibitory action of pergolide on "sensitive" SNC DA neuron firing. $\bar{x}\pm s$. $^{*}P<0.01$ *vs* **control.**

The depression caused by pergolide was readily attenuated by spiperone in the order of 0.25 mg kg^{-1} (Fig 2). These results indicate that pergolide possesses D_2 receptor agonist properties.

When Sch-23390 (1 — 2 mg kg^{-1}) was given iv following pergolide 20 μg kg^{-1} or more, the pergolide-caused rate reduction was also attenuated and the pretreatment firing

rates were regained (Fig 2).

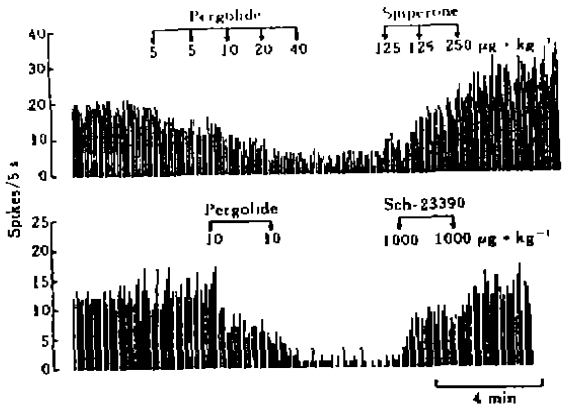

Fig 2. Pergolide-induced inhibition of SNC DA neuron firing was attenuated by spiperone and Sch-23390.

There were 3/14 rats insensitive to pergolide ($ID_{50} > 40$ μg kg^{-1}) (Fig 3). Even when the cumulative dose reached 160 μg kg^{-1}, firing activity was reduced by 53.9 %, while by 88.4 % in "sensitive" group with the cumulative dose of 80 μg kg^{-1}.

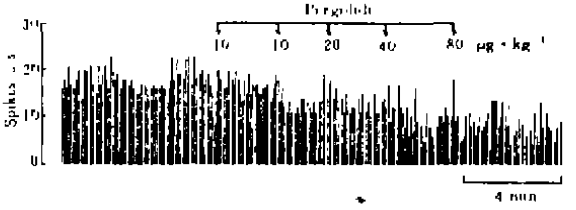

Fig 3. Response of "insensitive" SNC DA cell in paralyzed rat to iv pergolide.

Effects of bromocriptine on D_2 receptors

In another 6 rats, bromocriptine did not affect the firing rate (< 20 % change *vs* baseline) until the cumulative doses reached 6 or 12 mg kg^{-1} (inhibitory rate was 32.9 % and 79.2 %, respectively) (Fig 4).

ID_{50} of bromocriptine was 7.8 mg kg^{-1} (95 % fiducial limits, 3.3 — 18.5 mg kg^{-1}), which was approximately 650 times more than that of pergolide. However, different from that of pergolide, the inhibition induced by bromocriptine was not always attenuated by spiperone.

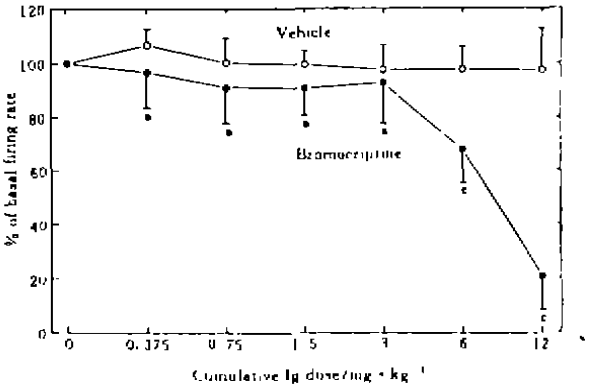

Fig 4. Inhibitory action of bromocriptine on SNC DA neuron firing. $n = 6$, $\bar{x} \pm s$. $^*P > 0.05$, $^*P < 0.01$ *vs* control.

In the control test ($n = 6$), vehicle induced only a small fluctuation of firing with no marked alteration *vs* baseline (Fig 4).

DISCUSSION

In the present work, the effects of pergolide and bromocriptine were compared using single unit recordings. In respect of ID_{50} values, pergolide was about 650 times more potent than bromocriptine. Among the DA cells recorded, a few cells were tested insensitive to pergolide. This phenomenon might be concerned with the different targets of DA cell projection. Substantial evidence indicated that there existed a small portion of SNC DA neurons terminating in cortical regions[10,11]. This subpopulation, in contrast to those innervating the striatum, was less sensitive to D_2 receptor agonists due to its lack of autoreceptors[12].

Pergolide and bromocriptine are 2 ergot derivatives used in clinic currently. This study showed that the inhibition of DA cell firing induced by pergolide was readily attenuated by the D_2 receptor antagonist spiperone.

However, bromocriptine's interaction with D_2 subtype was either reversible or irreversible by spiperone. This was consistent with others' observations[9] and ascribed to the peptide chain of bromocriptine which was capable of forming reactive intermediates to interact with accessory binding sites. Pergolide, an ergot derivative without peptide chain, induced agonist-reversible depression.

Pergolide, a dominant D_2 receptor agonist, has been discovered holding D_1 receptor agonist trace as well[13]. In our experiments, subsequent to the inhibition induced by pergolide, a large dose of a selective D_1 receptor antagonist Sch-23390 obviously attenuated the inhibition and returned the firing rates to the pretreatment baseline. This result indicates that pergolide in the previous doses acts not only on autoreceptors (D_2) but also on D_1 receptors which is postsynaptic to nigral DA neurons. This deduction is reasonable because the inhibitory effects of DA receptor agonists which were mediated through autoreceptors were not affected by Sch-23390 1 mg kg^{-1} or more with electrophysiological methods[14,15]. Furthermore, the similar phenomenon was observed with apomorphine, a D_1/D_2 mixed receptor agonist[14]. After a cumulative dose of 128 $\mu g\ kg^{-1}$ of apomorphine was given, Sch-23390 1 mg kg^{-1} significantly attenuated the apomorphine-induced suppression on firing rate. Sch-23390 4 mg kg^{-1} blocked the inhibitory effects of 32 $\mu g\ kg^{-1}$ of apomorphine. These effects result from postsynaptic D_1 receptors, which may locate in striatum.

In conclusion, the studies support that pergolide is a more potent D_2 receptor agonist than bromocriptine, and may have greater potential use to cure diseases associated with DA system. It is also suggested that pergolide may activate D_1 receptors *in vivo*, although its mechanism needs further study.

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培高利特对黑质多巴胺神经元放电活动的激动作用

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目的：研究 D_2受体激动剂培高利特(pergolide，Per)对大鼠黑质多巴胺(DA)神经元放电活动的影响，并与溴隐亭(bromocriptine，Bro)作比较，同时验证 Per 在整体动物有无 D_1激动剂性质。**方法**：胞外单细胞电活动记录技术。**结果**：二个药物均能抑制敏感及不敏感的 DA 神经元自发放电活动。Per 的 ID_{50}值为11.9 $\mu g\ kg^{-1}$，而 Bro 为7.8 $mg\ kg^{-1}$，Per 比后者强很多。选择性 D_2受体拮抗剂螺哌隆(spiperone，0.25 $mg\ kg^{-1}$) 或者选择性 D_1受体拮抗剂 Sch-23390 (1—2 $mg\ kg^{-1}$)可以减弱放电抑制。然而 Bro 引起的放电抑制并不都能为 spiperone 所减弱。**结论**：Per 在整体动物有很强的 D_2受体激动剂作用，比 Bro 强650倍，也有弱的 D_1受体激动剂的性质。

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关键词 培高利特；溴隐亭；螺哌隆；黑质；多巴胺受体；电生理学

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Effects of toquipidine on ionic channels of cultured embryonic *Xenopus laevis* myoblasts and neurons

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AIM: To study the effects of toquipidine (1-*p*-methyl-phenyl-2-(α-piperidinoacetyl)-1, 2, 3, 4-tetrahydroisoquinoline hydrochloride, Toq), a new anti-arrhythmic agent first synthesized in China, on ionic channels. **METHODS**: Ionic channel currents were recorded by whole-cell patch clamp technique in cultured embryonic *Xenopus laevis* myoblasts and neurons. **RESULTS**: Toq (0.1, 1, 10, and 100 $\mu mol\ L^{-1}$) caused a concentration-dependent inhibition of the Na^+ currents with IC_{50} 7.2 $\mu mol\ L^{-1}$ (5.3 — 9.8 $\mu mol\ L^{-1}$). Toq (10 $\mu mol\ L^{-1}$) also suppressed the high-voltage-activated Ca^{2+} currents in neurons. But the steady-state outward K^+ currents in myoblasts were activated by Toq (10 $\mu mol\ L^{-1}$).

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