中国药理学报 *Acta Pharmacologica Sinica* 1990 May; **11** (3) : 196-200

Effects of four dopamine agonists on *l*-tetrahydropalmatine-induced analgesia and electroacupuncture analgesia in rabbits

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ABSTRACT The effects of icv 4 dopamine (DA) agonists on analgesia caused by iv *l*-tetrahydropalmatine (THP) 8 mg/kg or by electroacupuncture (EA) were studied by using the potassium iontophoretic dolorimetry in rabbits. The results showed that both THP-induced analgesia and EA analgesia were markedly attenuated by icv of DA or apomorphine (Apo), 2 mixed D_1 / D_2 agonists. Similar results were obtained when SKF-38393, a selective D_1 agonist, was applied. On the contrary, quinpirole hydrochloride (Qui), a selective D_2 agonist, was found to enhance the analgesic action of THP or EA. However, DA, Apo, SKF-38393 or Qui *per se* did not influence the baseline pain threshold. All these observations indicate that functional alterations in DA receptor activities may be involved in THP-induced analgesia and EA analgesia, in which D_1 and D_2 subtype receptors exert different roles.

KEY WORDS *l*-tetrahydropalmatine; berbines; dopamine receptors; dopamine; apomorphine; SKF-38393; quinpirole; acupuncture; analgesia

Our previous studies have shown that *l*-tetrahydropalmatine (THP) and its analogues tetrahydroberberine (THB) and *l*-stepholidine (SPD) possess a potentiating effect on electroacupuncture (EA) analgesia in rabbits[1,2]. Based on the results of biological, behavioral and electrophysiological experiments, it suggested that THP and its analogues are a new type of dopamine (DA) receptor antagonists[3,4]. On the other hand, involvement of dopaminergic mechanisms in acupuncture analgesia has been also demonstrated by several studies[5,6]. Hence it was hypothesized that the functional alterations of central DA receptors could be involved in the potentiation of EA analgesia by THP and its analogues. In order to corroborate this hypothesis, we carried out a comparative evaluation of the influences of intracerebroventricular injection (icv) of 2 mixed DA agonists DA and apomorphine (Apo)[7,8] on the analgesia caused by THP or by EA. In addition, it was hoped to determine whether D_1 or D_2 receptor was associated with the analgesic actions of THP and EA, by using icv of SKF-38393 and quinpirole hydrochloride (Qui, LY-171555) which are generally accepted as being selective D_1 and D_2 agonists respectively[7,9,10].

Received 1989 Jul 6 Accepted 1989 Dec 22

MATERIALS AND METHODS

Rabbits of both sexes weighing 2.2 ± SD 0.4 kg were used.

Nociceptive testing Potassium iontophoresis method was used to measure pain threshold. The rabbit was restrained in a wooden box with the head and limbs exposed to allow free movement. K^+ was led into the skin of the ear by direct current as a nociceptive stimulus. The defensive reaction of the forelimbs and head was chosen as a criterion of pain response. The minimal value of electric current (mA) to elicit a pain response as demonstrated on a galvanometer was recorded as pain threshold.

The pain thresholds were measured at 5- or 10-min intervals before and after drug or EA administration for 100 min.

Electroacupuncture Unilateral "Hegu" point (the dorsum of the front paw, between the 1st and 2nd metacarpals) and "Waiguan" point (the dorsum of the foreleg, between the radius and ulna, 12 cm above the wrist joint) of each rabbit were needled and stimulated with an electrical stimulator (Model G6805).

The frequency range was 2–4 Hz and the intensity was sufficient to elicit local muscle contraction (5–10 mA). EA lasted 30 min.

Drugs and injections *l*–Tetrahydropalmatine sulfate injection (THP, Zhanjiang Pharmaceutical Co) was injected iv at a dose of 8 mg / kg[1,2]. Dopamine hydrochloride (DA, Sigma), apomorphine hydrochloride injection (Apo, Shenyang Pharmaceutical Co), SKF–38393 (Smith Kline and French) and quinpirole hydrochloride (Qui, EliLilly) were bilaterally administered icv via the stainless steel cannulae (0.9 mm outer diameter, 0.6 mm inner diameter) implanted into both lateral cerebroventricles according to Sawyer's atlas (AP_0, L or R_3, $H_{6.5}$). DA, SKF–38393 and Qui were dissolved in 0.9% saline. Icv volume was 20 μl / ventricle in 2 min. At the end of each experiment the position of the cannula was verified histologically.

Statistics For each group, $\bar{x} \pm SD$ were calculated and the significance between means compared using the 2 tailed *t*–test.

RESULTS

Having obtained at least 3 baseline pain threshold values among which differences were less than 0.3 mA, 4 DA agonists were injected icv respectively to observe their effects on baseline pain threshold and on the analgesic actions of THP and EA.

Effects of DA agonists on baseline pain threshold Icv of all these doses didn't affect the baseline pain threshold (Tab 1). The rabbits kept calm without any marked behavioral abnormality after these DA agonists except Qui. Qui was found to cause automatic movements of the heads and limbs at the high dose of 100 μg / ventricle but not at lower doses of 25 and 50 μg / ventricle.

Effects of DA agonists on THP–induced analgesia The results were shown in Tab 2. THP 8 mg / kg iv increased the pain threshold. Its effect arose at 5 min after administration and lasted about 20 min. When THP was administered concurrently with icv DA or Apo 100 μg / ventricle, there was a significant decrease in THP–induced analgesia. The antagonizing effects of DA were marked at 5 ($P < 0.01$,) and 10 min ($P < 0.05$), while the effect of Apo was significant only at 5 min ($P < 0.05$), suggesting that the antagonizing effect of Apo on THP–induced analgesia seems to be slightly weaker than that of DA. SKF–38393 was given icv 20 min prior to THP administration. At the dose of 50 μg / ventricle SKF–38393 significantly attenuated the analgesic action of THP. SKF–38393 25 μg / ventricle exerted a more potent attenuating effect, while 100 μg manifested an insignificant influence on THP–induced analgesia (data were not shown). When 50 μg dose of SKF–38393 was injected concurrently with THP, it showed no effect on THP–induced analgesia (data were not shown). These observations indicated that SKF–38393 had an attenuating effect on THP–induced analgesia, which was dependent on the dose and the time of administration. On the contrary, Qui, when icv injected concurrently with THP, potentiated the analgesic action of THP. The data obtained from Qui 50 μg / ventricle were listed in Tab 2. The linear regression analysis between the dose of Qui and the pain threshold was carried out at 5 min after combined medications of THP and Qui. The doses of 25, 50 and 100 μg / ventricle yielded changes in pain threshold of 1.44 ± 0.40, 2.65 ± 0.82 and 3.08 ± 0.88 mA ($n = 8$), respectively. The analysis showed a positive correlation ($Y = 3.93 \log X - 4.06$, $r = 0.9999$, $P < 0.001$), suggesting that Qui produced a dose–dependent enhancement of THP–induced analgesia. On the other hand, Qui 100 μg / ventricle given concurrently with THP did not cause automatic movements of the head and limbs which occurred when Qui was injected alone.

Effects of DA agonists on EA analgesia By using the same methods, we studied the effects of 4 DA agonists DA, Apo, SKF–38393 and Qui on EA analgesia and ob-

Tab 1. Effects of bilateral icv 4 dopamine (DA) agonists dopamine, apomorphine (Apo), SKF-38393 and quinpirole (Qui) on the baseline pain threshold of rabbits. $\bar{x} \pm SD$, $n=4$. $^*P>0.05$ *vs* **control.**

Compound	μg / ventricle	Pain threshold (mA) Control	5 min	10 min	30 min
DA	100	0.25±0.10	0.28±0.17*	0.28±0.13*	0.28±0.15*
Apo	100	0.43±0.26	0.45±0.31*	0.48±0.22*	0.40±0.20*
SKF-38393	25	0.25±0.13	0.40±0.14*	0.32±0.10*	0.40±0.16*
	50	0.41±0.24	0.44±0.30*	0.46±0.27*	0.42±0.21*
	100	0.37±0.21	0.41±0.27*	0.39±0.19*	0.43±0.24*
Qui	25	0.58±0.34	0.68±0.49*	0.53±0.29*	0.50±0.24*
	50	0.52±0.15	0.44±0.19*	0.44±0.20*	0.51±0.17*
	100	0.58±0.32	0.43±0.26*	0.43±0.13*	0.55±0.24*

tained results essentially similar to those seen above.

As showed in Tab 3, EA produced a marked elevation of pain threshold, which emerged at 10 min after the beginning of EA and continued for nearly 20 min after the stopage of EA. At 100 μg / ventricle dose, DA and Apo, icv at 10 min after the beginning of EA, significantly antagonized EA analgesia respectively. The antagonisms appeared immediately after DA or Apo injection and lasted about 40 min. The data indicated that the antagonizing effects of DA and Apo on EA analgesia were quite similar to each other in the intensity and duration. SKF-38393 was injected icv at 10 min before the beginning of EA. SKF-38393 50 μg / ventricle markedly antagonized EA analgesia. Similar results were seen with 25 μg / ventricle (data were not shown). In contrast, Qui 25 and 50 μg / ventricle icv at 10 min after the beginning of EA potentiated EA analgesia. Qui 25 μg / ventricle merely potentiated the analgesic duration of EA and did not influence the potency (data were not shown). The reason for failing to observe the effect of Qui 100 μg / ventricle on EA analgesia was that the resulted automatic movements of the head and limbs interfered with the pain measurement.

DISCUSSION

The present results showed that the analgesic action of THP was antagonized by either DA or Apo, 2 mixed D_1/D_2 agonists[7,8]. Together with other results showing that THP exerts an antagonistic effect to central DA receptors[3,4], they would imply that THP-induced analgesia is mediated via the blockade of central DA receptors. Concerning the influences of DA and Apo on EA analgesia, our results are in agreement with the previous findings[5] indicating that acupuncture analgesia was significantly antagonized by icv Apo 100 μg / ventricle in rabbits. However, icv Apo 4 μg / ventricle potentiated EA analgesia in mice[6]. Some of these conflicting results may be attributed to a difference in the dose of Apo[11]. The same similar influences of these 2 DA agonists on both THP-induced analgesia and EA analgesia prompt us to hypothesize that THP, via blocking central DA receptors, produces its analgesia, and thereby exhibits a potentiating effect to EA analgesia.

There is a clear distinction between D_1 and D_2 receptors in pain modulation[12-15]. These findings were comfirmed by our observation that the effects of selective D_1 agonist SKF-38393[7,9] and selective D_2 agonist Qui[7,10] were quite different on either THP-induced analgesia or EA analgesia. It was worth noting that the effect of SKF-38393 was considerably similar to those of DA and Apo on the analgesic actions of THP and EA. It suggests that central D_1 receptor most likely plays a dominant role in THP- or EA-in-

Tab 2. Effects of bilateral icv 4 DA agonists on analgesia induced by iv *l*– tetrahydropalmatine (THP) 8 mg / kg in rabbits. DA, Apo and Qui were injected at 2–3 min before THP administration and SKF–38393 was injected at 20 min before administration. $n=8$, except DA $n=10$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* corresponding THP + NS (normal saline).

THP +	μg / ventricle	Changes of pain threshold after *l*–tetrahydropalmatine injection (mA) 5 min	10 min	15 min	20 min	30 min	40 min	50 min
NS		1.08±0.76	0.84±0.74	0.49±0.62	0.24±0.21	0.24±0.24	0.23±0.30	0.19±0.12
DA	100	0.16±0.18***	0.20±0.42**	0.12±0.39*	0.10±0.22*	0.12±0.30*	0.16±0.40*	0.07±0.27*
Apo	100	0.23±0.54**	0.25± 0.43*	0.14±0.32*	0.14±0.33*	0.13±0.32*	0.07±0.14*	0.14±0.20*
NS		0.89±0.22	0.93±0.21	0.74±0.25	0.54±0.18	0.49±0.27	0.34±0.27	0.26±0.32
SKF–38393	50	0.36±0.32***	0.26±0.23***	0.17±0.10***	0.13±0.09***	0.09±0.08***	0.08±0.07**	0.05±0.05*
NS		0.84±0.30	1.08±0.44	0.71±0.42	0.61±0.23	0.25±0.23	0.14±0.21	0.09±0.20
Qui	50	2.65±0.82***	2.30±0.64***	2.29±0.67***	1.63±0.93***	1.00±0.59***	0.64±0.34***	0.50±0.32***

Tab 3. Effects of bilateral icv 4 DA agonists on electroacupuncture (EA) analgesia in rabbits. DA, Apo and Qui were injected at 10 min after EA beginning, and SKF–38393 was injected at 10 min before EA beginning. $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* corresponding EA + NS.

EA +	μg / ventricle	*n*	Changes of pain threshold after electroacupuncture beginning (mA) 10 min	20 min	30 min	40 min	50 min	60 min	70 min
NS		8	0.45±0.22	0.66±0.21	0.54±0.15	0.43±0.21	0.33±0.19	0.20±0.13	0.09±0.15
DA	100	8	0.44±0.17*	0.21±0.16***	0.10±0.11***	0.05±0.08***	0.09±0.10***	0.13±0.16*	0.03±0.04*
Apo	100	8	0.43±0.12**	0.20±0.26***	0.06±0.25***	0.04±0.20***	0.05±0.21**	0.03±0.19*	−0.01±0.14***
NS		9	0.40±0.30	0.84±0.46	0.72±0.54	0.60±0.58	0.41±0.43	0.28±0.40	0.26±0.35
SKF–38393	50	8	0.28±0.30*	0.10±0.14***	0.14±0.20***	0.16±0.15*	0.05±0.14**	0.05±0.11*	0.06±0.12*
NS		9	0.43±0.21	0.62±0.19	0.39±0.09	0.30±0.12	0.18±0.15	0.09±0.13	−0.01±0.11
Qui	50	8	0.51±0.17*	1.60±0.75***	1.48±0.56***	1.18±0.59***	0.85±0.49***	0.70±0.52***	0.53±0.53***

duced analgesia. The competitive binding test revealed that THP and its analogues displayed a preferential affinity toward the central D_1 receptor[4], which provided the above suggestion with some supports. On the other hand, the potentiation of THP–induced analgesia by Qui may be attributed to the interaction between the blockade of D_1receptor by THP and the activation of D_2 receptor by Qui since a reciprocal relationship between $D_1$and D_2 receptors in pain modulation has been proposed by other workers[15].

Studies involving the influences of DA agonists on baseline pain threshold have produced conflicting results. Apo and SKF–38393 were without effects when given icv [5, 6, 12, 14], which is consistent with the present results. However, icv LY–141865, an isomer of Qui, increased the baseline pain threshold in mice[12]. The discrepancy between this result and our current data showing that Qui was inactive may be attributed to the animal and the isomeric differences.

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四种多巴胺受体激动剂对左旋四氢巴马汀和电针镇痛的影响

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提要 在兔 K^+透入测痛模型上, 双侧 icv 多巴胺(DA)受体广谱激动剂 DA 和去水吗啡(Apo)以及D_1受体选择性激动剂 SKF-38393 对抗 iv 左旋四氢巴马汀(THP) 8 mg/kg 或电针一侧"合谷"和"外关"穴引起的镇痛作用. 相反, 双侧 icv D_2受体选择性激动剂喹吡罗则呈剂量相关地加强 THP 或电针的镇痛作用. 结果提示, THP 和电针两者的镇痛作用皆与中枢 DA 受体活动有关, 其中 D_1 和 D_2受体的作用可能不同.

关键词 左旋四氢巴马汀; 小檗因类; 多巴胺受体; 多巴胺; 去水吗啡; SKF-38393; 喹吡罗; 针刺; 镇痛

中国药理学报 *Acta Pharmacologica Sinica* 1990 May; **11** (3) : 200-203

Simultaneous electric activities of pain-excitation and pain-inhibition neurons in nucleus parafascicularis of thalamus in rats during acute morphine tolerance

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Received 1989 May 08 Accepted 1990 Jan 12

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ABSTRACT When acute morphine-tolerated rat was administered by ip morphine (10 mg/kg) which was effective before the acute tolerance to morphine, both the inhibitory effect of morphine on the electric discharges of pain-excitation neurons (PEN) in nucle-