BS. Evidence for the absence of impulse-regulating somatodendritic and synthesis modulating nerve terminal autoreceptors on subpopulations of mesocortical dopamine neurons. Neuroscience 1984; 12: 1—16.

13 Goldstein M, Lieberman A, Lew JY, Asano T, Rosenfeld MR, Makman MH. Interaction of pergolide with central dopaminergic receptors. Proc Natl Acad Sci USA 1980; 77: 3725—8.

14 Napier TC, Givens BS, Schulz DW, Bunney BS, Breese GR, Mailman RB. SCH23390 effects on apomorphine-induced responses of nigral dopaminergic neurons. J Pharmacol Exp Ther 1986; 236: 838—45.

15 Carlson JH, Bergstrom DA, Walters JH. Neurophysiological evidence that D-1 dopamine receptor blockade attenuates postsynaptic but not autoreceptor-mediated effects of dopamine agonists. Eur J Pharmacol 1986; 123: 237—51.

423-427

培高利特对黑质多巴胺神经元放电活动的激动作用

张雪翔，金国章，魏月芳

R965.2

（中国科学院上海药物研究所，上海 200031，中国）

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。

10

关键词 培高利特；溴隐亭；螺哌隆；黑质；多巴胺受体；电生理学

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Sep; 16 (5): 427—439

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

ZHENG Ping, CHEN Gong[1], SHI Nian-Ci, ZHOU Si-Ping, LU Ying-Qing, YAO Ming-Hui, HE Zeng-You[2] (*Department of Pharmacology, School of Basic Medical Science, Shanghai Medical University, Shanghai 200032; [1]Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031; [2]Yangzhou Pharmaceutical Factory, Yangzhou 225001, China*)

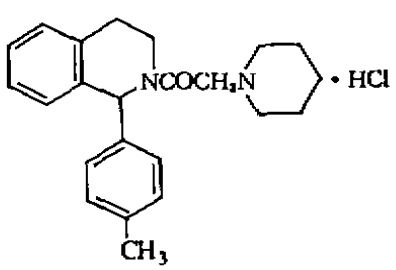
AIM: To study the effects of toquipidine (1-*p*-methyl-phenyl-2-(α-piperidinoacetyl)-1, 2, 3, 4-tetrahydroisoquinoline hydrochloride, Toq), a new anti-arrhythmic agent first synthesized in China, on ionic channels. **METHODS**: Ionic channel currents were recorded by whole-cell patch clamp technique 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 $\mu mol\ L^{-1}$).

Received 1994-01-10 Accepted 1995-01-14

CONCLUSION: Toq blocked the Na^+ and Ca^{2+} channels and opened the steady-state outward K^+ channels.

KEY WORDS toquipidine; isoquinolines; electrophysiology; cultured cells; muscles; neurons; sodium channels; calcium channels; potassium channels; *Xenopus laevis*

Toquipidine (1-*p*-methyl-phenyl-2-(α-piperidinoacetyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride, Toq) is a new antiarrhythmic agent first synthesized in China. *In vivo*, Toq exerted high anti-arrhythmic effects against aconitine arrhythmia in rats with a lower acute toxicity than aprindine[1]. In isolated guinea pig ventricular muscles, Toq suppressed the maximal upstroke velocity and the amplitude of action potentials with shortened action potential duration. Toq may have effects on more than one ionic channel, but the precise actions were not elucidated. In the present study, the effects of Toq on ionic channels were investigated by standard whole-cell patch clamp technique in cultured embryonic *Xenopus laevis* myoblasts and neurons to elucidate the mechanisms of the anti-arrhythmic effect of Toq.



Toquipidine

MATERIALS AND METHODS

Cell culture Embryonic *Xenopus laevis* myoblasts and neurons were cultured[2], after the neural tube and the associated myotomal tissue of 1-d old *Xenopus* embryos (stage 19 — 22) were dissociated, the cells were plated on glass coverslips for culture. The cells were used within 2—5 d after culture.

Electrophysiological recording A conventional whole-cell GΩ seal recording was used[3]. The electrodes were pulled in 2 stages and fire-polished on a microforge. The currents were recorded with a patch clamp amplifier (V Pan-tani, Yale University, USA). For recording Na^- currents (I_{Na}), the external solutions contained NaCl 115, $CaCl_2$ 5, KCl 2.5, HEPES 10 (mmol L^{-1}), pH 7.4. The pipette was filled with a solution containing CsCl 110, Cs_2 BAPTA (1,2-bis (2-aminophenoxy) ethane *N*, *N*, *N'*, *N'*-tetraacetic acid) 2, $MgCl_2$ 2, HEPES 10 (mmol L^{-1}), pH 7.4. For recording Ca^{2-} currents (I_{Ca}), the bath solution contained TEA (tetraethylammonium)-Cl 115, $CaCl_2$ 5, TTX (tetrodotoxin) 0.001, HEPES 10 (mmol L^{-1}), pH 7.4. The pipette solution was the same as that for recording I_{Na}. For recording K^+ currents (I_k), the pipette was filled with a solution containing KCl 110, K_2 egtazic acid 1; $MgCl_2$ 2, HEPES 10 (mmol L^{-1}), pH 7.4. The bath solution was the same as that for recording I_{Na}. Data were digitized by an AD converter and processed by a computer.

Data analyses Statistical analyses were made by *t* test.

Drug Toquipidine (white powder, mp 216 — 8 C) provided by Department of Medicinal Chemistry, Shanghai Medical University, was dissolved in the test solution to obtain the final concentration indicated in the text.

RESULTS

1 Identification of ionic currents The current-voltage relationship of I_{Na} (activated at about −65 mV, peaked at −20 mV and reversed at +40 mV), I_k (activated at about −40 mV and saturated at about +40 mV) and I_{Ca} (activated at about −10 mV, peaked at about +10 mV and reversed at about +50 mV) were similar to those reported in the literatures[4—6]. When the membrane of the cultured embryonic Xenopus myoblast was clamped from the holding potential of −80 mV to +10 mV, an inward current with fast

activation and inactivation was seen. This inward current was inactivated by TTX 1 μmol L^{-1}. A steady-state outward current was evoked by an 120-ms depolarization from the holding potential of −80 mV to +10 mV in the myoblast. This outward current was inactivated by TEA 115 mmol L^{-1}. When the membrane of the neuron was clamped from the holding potential of −80 mV to +10 mV, an inward current with slow activation and inactivation was seen. This inward current was inhibited by verapamil (Ver) 100 μmol L^{-1}. The above-mentioned characteristics verified that these currents were the inward sodium current (I_{Na}), the steady-state outward potassium current (I_k) and the inward calcium current (I_{Ca}), respectively.

2 Effects of Toq on ionic channels

2.1 Effects of Toq on I_{Na}

2.1.1 Concentration dependence I_{Na} was evoked by a 20-ms depolarization to −10 mV at a frequency of 0.1 Hz. The holding potential was −80 mV. Toq caused a concentration-dependent inhibition of I_{Na} with IC_{50} 7.2 μmol L^{-1}(5.3—9.8 μmol L^{-1}) (Tab 1).

Tab 1. Concentration dependence of Toq-induced blockage of Na^+ currents in cultured embryonic *Xenopus* myoblasts. I_{Na} was expressed as $I/I_{control}$. n=5—6, $\bar{x}\pm s$.

Toquipidine/ μmol L^{-1}	$I/I_{control}$
0.1	0.92±0.07
1	0.64±0.13
10	0.45±0.12
100	0.25±0.15

2.1.2 Time course I_{Na} was evoked by a 20-ms depolarization to −10 mV at a frequency of 0.1 Hz. The holding potential was −80 mV. After application of Toq 10 μmol L^{-1}, the depression of I_{Na} developed rapidly and persisted in the presence of Toq, but the effects were reversed by the washout of the drug (Fig 1).

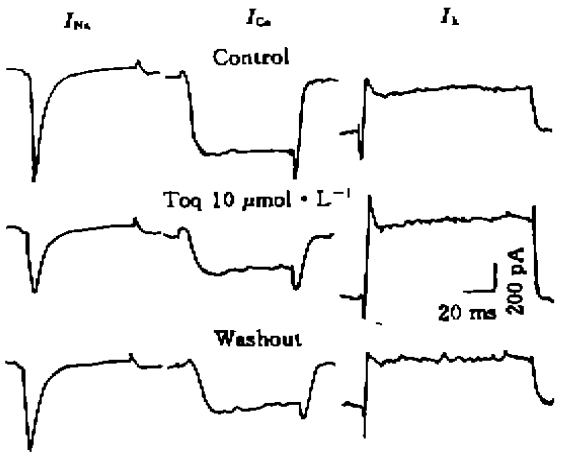


Fig 1. Effects of Toq 10 μmol L^{-1} on ionic channels.

2.2 Effects of Toq on I_k The steady-state outward I_k were evoked by a 120-ms depolarization to +10 mV at a frequency of 0.1 Hz. The holding potential was −80 mV. Toq 10 μmol L^{-1} increased I_k and the effects were reversed by the washout of the drug (Fig 1, Tab 2).

Tab 2. Effects of Toq 10 μmol L^{-1} on I_k and I_{Ca}. n=6, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

	I_k(nA)	I_{Ca}(nA)
Control	0.33±0.20	0.37±0.10
Toq 10 μmol L^{-1}	0.75±0.45^b	0.18±0.07^c

2.3 Effects of Toq on I_{Ca} High-voltage-activated I_{Ca} were elicited by a 20-ms depolarization to 10 mV at a frequency of 0.1 Hz. Toq 10 μmol L^{-1} markedly decreased I_{Ca} and the effects were partially decreased by the washout of the drug for 5 min (Fig 1, Tab 2).

DISCUSSION

The present study showed that Toq had the blocking effect on I_{Na}. The threshold

concentration of Toq to inhibit I_{Na} was close to its anti-arrhythmic concentration in rats, suggesting that the blocking effect on I_{Na} may be one of the mechanisms of the anti-arrhythmic effect of Toq.

Embryonic *Xenopus* neurons have been shown to display Ca^{2+} currents that correspond to the low-voltage-activated (T-type) and high-voltage-activated (HVA) forms described in other excitable cells[6]. T-typed Ca^{2+} current was activated at voltages positive to −50 mV and was selectively blocked by Ni^{2+} 200 μmol L^{-1}. HVA I_{Ca} was observed at voltages positive to −10 mV and was sensitive to Ver. In this study, we observed the effects of Toq on HVA I_{Ca}. The results showed that Toq at therapeutic concentration (10 μmol L^{-1}) suppressed not only I_{Na}, but also I_{Ca}. This result was similar to that of aprindine, which blocked both the I_{Na} and I_{Ca} in frog atria[7]. The inhibitory effect of Toq on I_{Ca} may play a role in preventing the arrhythmias secondary to enhanced calcium influx.

Aprindine has been shown to depress the steady-state outward currents in frog atria, which resulted in the prolongation of the action potential duration[7]. Here, we found that Toq, rather than depressing I_k, increased I_k. This effect would make Toq to be more effective in shortening action potential duration in that the effects of Toq on I_{Ca} and I_k were both beneficial to the shortening.

On the basis of the present results, it is concluded that Toq has sodium and calcium channel blocking properties and steady-state outward potassium channel opening property. These properties play a predominant role in the anti-arrhythmic effect of Toq.

REFERENCES

1 Zhou ZS, Gu KJ, Shen XT, Chen ZH, Lin JB, Song GX. Syntheses of 1, 2-disubstituted tetrahydroisoquinoline derivatives and their antiarrhythmic effects. Acta Acad Med Shanghai 1987; **14**: 15—20.

2 Feng TP, Dai ZS. The neuromuscular junction revisited: Ca^{2+} channels and transmitter release in cholinergic neurons in *Xenopus* nerve and muscle cell culture. J Exp Biol 1990; **153**: 129—40.

3 Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 1981; **391**: 85—100.

4 Koumi SI, Sato R, Hisatome I, Hayakawa H, Okumura H, Katori R. Disopyramide block of cardiac sodium current after removal of the fast inactivation process in guinea pig ventricular myocytes. J Pharmacol Exp Ther 1992; **261**: 1167—74.

5 Fan Z, Hiraoka M. Depression of delayed outward K^+ current by Co^{2+} in guinea pig ventricular myocytes. Am J Physiol 1991; **261**: C23—C31.

6 Barish ME. Voltage-gated calcium currents in cultured embryonic Xenopus spinal neurones. J Physiol (Lond) 1991; **444**: 523—43.

7 Gilmour RF Jr, Chikharev VN, Jurevichus JA, Zacharov SI, Zipes DP, Rosenshtraukh LV. Effect of aprindine on transmembrane currents and contractile force in frog atria. J Pharmacol Exp Ther 1981; **217**: 390—6.

甲苯喹哌对培养爪蟾胚胎肌细胞和神经细胞离子通道的作用

郑 平, 陈 功[1], 史念慈, 周思平, 鲁映青, 姚明辉, 何曾佑[2] (上海医科大学基础医学院药理教研室, 上海200032; [1]中国科学院上海生理研究所, 上海200031; [2]扬州制药厂, 扬州225001, 中国)

目的: 研究新型抗心律失常药甲苯喹哌对离子通道的作用. **方法**: 通过膜片钳技术记录培养爪蟾胚胎肌细胞和神经细胞全细胞离子通道电流. **结果**: 甲苯喹哌(0.1, 1, 10, 100 μmol L^{-1})可浓度依赖性地抑制肌细胞的钠通道, 其 IC_{50}为7.2 μmol L^{-1}(5.3—9.8 μmol L^{-1}). 甲苯喹哌(10 μmol L^{-1})可抑制神经细胞的高电压激活的钙通道. 然而, 肌细胞上的稳态外向钾电流却受到甲苯喹哌(10 μmol L^{-1})的激活. **结论**: 甲苯喹哌抑制钠、钙通道, 但激活稳态外向钾通道.

关键词 甲苯喹哌; 异喹啉; 电生理学; 培养的细胞; 肌; 神经元; 钠通道; 钙通道; 钾通道; 有爪蟾蜍

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Sep; 16 (5): 431—434

Expressions of preproenkephalin mRNA during electroacupuncture analgesia enhanced by fenfluramine[1]

LI Xiao-Yan, ZHU Chong-Bin, ZHU Yan-Hua, XU Shao-Fen (*State Key Laboratory of Medical Neurobiology, Shanghai Medical University, Shanghai 200032, China*)

AIM: To study the changes of preproenkephalin (PPE) mRNA in rat brain in response to electroacupuncture (EA) combined with fenfluramine (Fen), a releaser of 5-HT. **METHODS:** *In situ* hybridization histochemistry technique was used to observe the expression of PPE mRNA in rat brain during EA analgesia potentiated by Fen. **RESULTS:** The greatest relative increase of PPE mRNA was seen in lumbar spinal cord (laminae I & II), nucleus raphe magnus, dorsal raphe nucleus, periaqueductal gray, interpeduncular nucleus, preoptic lateral area, amygdala nucleus and caudate-putamen ($P<0.01$, *vs* NS + EA). Moderate increases were found in lateral septal, preoptic medial area, hypothalamus ventromedial nucleus, lumbar spinal laminae III & IV ($P<0.05$, *vs* NS + EA). Thalamus showed no statistical significant change in PPE mRNA. **CONCLUSION:** The enhancing of PPE mRNA in relative brain nuclei is involved in potentiating action of Fen on EA.

KEY WORDS enkephalins; messenger RNA; fenfluramine; electroacupuncture; *in situ* hybridization; central nervous system

Serotonin (5-HT) is involved in and beneficial to electroacupuncture (EA) analgesia (EAA)[1,3]. Fenfluramine (Fen), a releaser of 5-HT, enhances acupuncture analgesia markedly[2]. The effect of Fen was related to endogenous opioid system[4,5] which may play a key role. However, the mechanism of potentiating effect of Fen on EAA is not clear. The present study was to observe the expression of PPE mRNA in EA with/without Fen in certain rat brain, thus to get some new understanding on the relationship between opioidergic and serotoninergic systems.

MATERIALS AND METHODS

Rats Sprague-Dawley rats (♂, $n=20$, 215±35 g) were bred by the Department of Experimental Animals, Shanghai Medical University.

Reagents Digoxigenin (Dig) RNA labelling Kit and Dig detection Kit were from Boehriger Mannheim; Restriction endonucleases Sac 1 and Hinf 1 RNAase were from Sino-American Biotechnology Company; Proteinase K was from Sigma; Other reagents were all AR.

Nociceptive test Nociceptive test was undertaken in quiet surrounding at 18 ℃. The rat tail was inserted subcutaneously with needles which were connected with pain test apparatus (Model WO-9E Pain Threshold Meter, Beijing). The least intensity of stimulating current that induced the tail flick was recorded as pain threshold. The pain threshold test was repeated thrice as the preadministration control, which ranged from 0.1 to 0.2 mA in normal rats.

EA EA was applied unilaterally at points (the needles were inserted 5 mm) of "Zu-San-Li" (ST 36, between muscle anterior tibialis and muscle extensor digitorum longus) and "Kun-Lun" (B60, in the depression between the tip of the external malleolus and tendo calcuneus) by EA apparatus (Model 6805-2, Shanghai with the dense-sparse frequency of wave and 20 V intensity, which provoked slight twitches of hindlimb.

Tissue preparation The rats were treated with (1) normal saline (NS) ip; (2) Fen 4.5 mg kg^{-1} ip

[1] Project supported by the National Natural Science Foundation of China, № 39370235.
Received 1994-05-18 Accepted 1995-06-05

(Fen); (3) EA for 20 min (NS+EA); (4) Fen 4.5 mg kg^{-1} ip for 10 min, then EA for 20 min (Fen+EA). After 10 h, rats were deeply anesthetized, and perfused intracardial with 4 % paraformaldelyde. The brain and spinal cord were sliced on freezing microtome (30 μm). The sections were stored in the modified cryoprotectant solution (500 mL phosphate buffer solution 0.1 mol L^{-1} pH 7.2, 300 g sucrose, 300 mL ethylene glycol, with the final volume adjusted to 1 L with H_2O) at −20 C.

***In situ* hybridization histochemistry method** For labelling PPE RNA probe with Dig and *in situ* hybridization were reported[6]. The sections were put into a sterile culture dish containing PBS 0.1 mol L^{-1} and rinsed, then placed in 1.5 mL epintorph with 500 ng L^{-1} PPE probe and hybridization buffer (50 % formamide, 5 × SSC, 2 % (w/v) blocking reagent, 0.02 % (w/v) SDS) and incubated at 37 C for 16 h. After hybridization, the sections were rinsed in 2 × SSC, treated with RNAase (20 μg L^{-1}) and rinsed 0.5 × SSC. Finally, immunological detection was proceeded and the sections were incubated in color solution (levamisole 0.24 g L^{-1}, nitroblue tetrazolium salt 0.34 g L^{-1}, 5-bromo-4-chloro-3-indolylphosphate toluidinium salt 0.18 g L^{-1} in NaCl 100 mmol L^{-1}, MgCl 50 mmol L^{-1} pH 9.5) for 6 h at 25 C. The section was mounted and examined under light microscope (Nikon).

Using computer-assisted image processing system (FG-100-AT, Imaging Technology Inc, and TV-Camera, RCA Inc, USA), the density of PPE mRNA positive neurons was measured by the gray level of cell image. Data were analyzed by *t*-test between 2 samples.

RESULTS

Effect of Fen on EAA Changes of pain threshold in NS group was observed for 90 min. When unilateral EA was applied, the pain threshold increased. There was no significant change in response of ip Fen 4.5 mg kg^{-1}. After Fen + EA, the pain threshold was further increased and prolonged *vs* NS+EA group ($P<0.01$), with the peak appearing at 30 min following EA (Fig 1).

Expression of PPE mRNA In NS group,

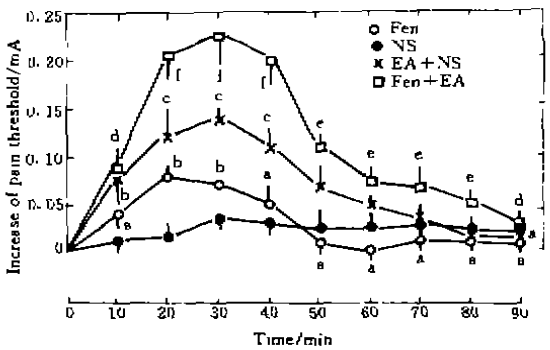


Fig 1. Effect of fenflulamine (Fen) of electroacupuncture analgesis (EAA). $n=5$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* NS group; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* EA group.

PPE mRNA positive neurons were seen in caudate putamen, accumben nucleus, preoptic area, periaqueductal gray, dorsal raphe nucleus, and lumbar spinal cord. In rats treated with Fen + EA, the highest increase of PPE mRNA content was seen in caudate putamen, amygadala nucleus, accumbens nucleus, preoptic lateral area, interpeduncular nucleus, periaqueductal gray, dorsal raphe nucleus, nucleus raphe magnus, and lumabr spinal cord (laminae I and II), compared with that of NS +EA group ($P<0.01$). Moderate increases of PPE mRNA content in Fen + EA group were detected in lateral septal, preoptic medial area, hypothalamus ventromedical nucleus, and lumbar spinal laminae III IV, compared with those of NS+EA ($P<0.05$). Thalamus showed little change in PPE mRNA *vs* NS group ($P>0.05$). In NS+EA group, there were increases in above areas, but the increases were less than those in Fen + EA group. In Fen group, the increase of PPE mRNA was seen in caudate putamen and amygdala nucleus, dorsal raphe nucleus, raphe magnus nucleus and lumbar spinal cord (laminea I and II) (Tab 1).

The neuroanatomical localizations of PPE-positive cells showed different degree

Tab 1. Density of PPE mRNA positive neurons in brain. ($n=4$, $\bar{x}\pm s$). $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* NS (normal saline); $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* Fen (fenfluramine); $^gP>0.05$, $^hP<0.05$, $^iP<0.01$ *vs* NS+EA (electroacupuncture).

	NS	FFM	NS+EA	FFM+EA
Telencephalon				
Caudate-putamen	10.9±1.3	17.7±1.5^b	25.1±3.3^c	50.5±3.5^{cfh}
Amygdala				
Lateral nucleus	12.0±2.3	20.5±0.7^b	30.7±1.0^c	47.0±2.6^{cfi}
Accumbens	12.3±1.0	13.2±1.0^d	31.6±1.3^c	48.6±1.8^{cfi}
Preoptic area				
Lateral area	10.5±1.7	13.0±2.0^d	29.2±1.2^c	46.8±2.5^{cfi}
Medial area	11.0±1.1	11.6±2.7^d	29.3±1.0^c	33.6±2.1^{cfh}
Lateral septal nucleus (dorsal part)	10.7±2.2	13.7±1.8^d	20.7±1.3^c	27.0±2.9^{cfh}
Diencephalon				
Hypothalamus				
Ventromedial nucleus	16.5±0.7	18.3±2.3^d	25.7±2.2^c	33.8±2.4^{cfh}
Mesencephalon				
Periaqueductal gray (ventral part)	8.0±1.1	10.0±1.4^d	15.8±2.1^c	27.5±2.8^{cfh}
Interpeducular nucleus (central)	16.0±1.0	19.0±2.3^d	23.0±2.1^c	50.8±3.0^{cfi}
Dorsal raphe nucleus	9.3±1.8	24.7±2.0^c	21.1±2.1^c	48.6±2.4^{cfi}
Pons/medulla				
Raphe magnus nucleus	15.1±1.8	21.6±1.1^b	15.3±2.4^c	43.1±3.0^{cfi}
Lumbar spinal cord				
Laminae I—II	17.2±1.4	26.0±1.1^b	31.1±1.3^c	48.1±3.0^{cfi}
Laminae III—IV	11.3±0.8	12.9±0.9^d	15.1±1.0^b	20.1±1.2^{cfh}

changes between Fen+EA group and NS+EA group in caudate putamen, interpeduncular nucleus, and lumbar spinal dorsal horn. The density of PPE mRNA was markedly increased in the brain treated with Fen+EA (Fig 2B, D, Plate 2) *vs* EA (Fig 2A, C). Marked change of PPE mRNA contents were seen in different layers of lumbar spinal cord. When Fen was combined with EA, laminae I showed labelled PPE neuron cells (Fig 2F). Whereas in EA group, the levels of PPE mRNA in lamina III and IV were low (Fig 2E).

DISCUSSION

In our results, the increase of PPE mRNA positive neurons in areasclosely related to nociceptive modulation such as caudate putamen, accumben nucleus, preoptic area, periaqueductal gray and the dorsal horn of spinal cord was seen in EA group. It is generally accepted that the opioidergic system play an important role in nociception and analgesia[5,8]. These results are in line with such a view.

Our previous work showed the fentanyl could further increase the Fen potentiating effect on EAA[2]. The finding that 10 h after EA combined with Fen, the levels of PPE mRNA significantly increased in areas mentioned above including dorsal raphe nucleus, nucleus raphe magnus and the dorsal horn of spinal cord, and the degree of increase was higher than that of EA or Fen along, further demonstrated that the opioidergic system was involved in Fen potentiation in EAA. The enhanced PPE mRNA content in above areas was related to after-effect of Fen potentiation on EAA and reflected the long-term or chronic readiness of a cell to synthesize PPE precursor which had lost.

Recently we reported that using autoradiographic technique, the increased density of opioid receptors induced by EA combined with Fen occurred in brain nuclei mentioned above[9]. These result are also strongly consistent, indicating the opioidergic system is involved in serotonin potentiation in EAA.

In summary, EA and EA combined with Fen all induced the increasing expression of PPE mRNA in certain central areas closely related to nociceptive modulation. The degree of expression by EA combined with Fen was far stronger than that of EA. The results suggested that the endogenous opioid peptides be involved in EAA potentiated by Fen.

REFERENCES

1 Han JS, Tang J, Ren MF, Zhou F, Fan SG, Qiu XC. Central neurotransmitters and acupuncture analgesia. Am J Chin Med 1980; 8: 331—48.

2 Huang LY, Sheng MP, Xu SF. Effect of fenfluramine on electroacupuncture analgesia. Acta Acad Med Shanghai 1987; 14: 440.

3 Han JS, Ton J, Fan SG. The contents of 5-hydroxytryptamine (5-HT) and morphine-like substance (MLS) in the brain and electroacupuncture analgesia (AA). Acta Physiol 1978; 30: 201—3.

4 Groppetti A, Parenti M, Dellavedova L, Tirone F. Possible involvement of endogenous opiates in the tolerance to the anorectic effect of fenfluramine. J Pharmacol Exp Ther 1984; 228: 446—53.

5 Harsing LG, Yang HY, Gorani S, Cowta E. Elevation of met-enkephalin and beta-endorphin hypothalamic content in rats receiving anorectic drugs: Differences between *D*-fenfluramine and *D*-amphetamine. Neuropharmacology 1982; 21: 141—6.

6 Li XY, Zhu CB, Yin X, Zhu YH, Xu SF. A free-floating method for in situ hybridization histochemistry using DIG labelled RNA probes. Acta Acad Med Shanghai 1995; 22: 79—82.

7 He LF. Involvement of endogenous opioid peptides in acupuncture analgesia. Pain 1987; 31: 99—121.

8 Han JS. High and low frequency electroacupuncture analgesia are mediated by different opioid peptides. Pain 1984; 2 Suppl: 369.

9 Li XY, Zhu CB, Zhu YH, Xu SF. Changes of opioid receptor in rat brain during electroacupuncture analgesia potentiated by fenfluramine. Acta Acad Med Shanghai 1994; 21 Suppl: 50—5.

431-434 12

氯氟拉明加强针刺镇痛时前脑啡肽原 mRNA 的表达

R965.2

李晓艳，朱崇斌，朱燕华，许绍芬

（国家医学神经生物重点实验室，上海医科大学神经生物教研室，上海200032，中国）

目的：研究大鼠脑内前脑啡肽原(PPE) mRNA的变化与5-HT的释放剂芬氟拉明加强针刺镇痛的关系. **方法**：用原位杂交组织化学法观察PPE mRNA的表达. **结果**：脊髓背角(I, II层)，中缝大核，中缝背核，中央灰质，脚间核，视前外侧区，杏仁核和尾壳核内的PPE mRNA的含量大量升高($P<0.01$, *vs* NS+EA)，而在隔外侧区，视前内侧区，下丘脑腹内侧核以及脊髓背角III—IV层的PPE mRNA的水平也有中等程度增高($P<0.05$, *vs* NS+EA)．但在丘脑却没有明显的变化．**结论**：PPE mRNA含量在与痛相关的区域里增高，是芬氟拉明加强针效的机制之一.

关键词 脑啡肽；信使RNA；芬氟拉明；电针；原位杂交；中枢神经系统

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Sep; 16 (5): 435—437

Effect of pineal body and melatonin on chemiluminescence of peritoneal macrophages via hypothalamus in rats

WEI Wei, DING Chang-Hai, XU Shu-Yun
(Institute of Clinical Pharmacology, Anhui Medical University, Hefei 230032, China)

AIM: To study whether the hypothalamus was a major relay for the action of melatonin (Mel) on peritoneal macrophage (PM∅) function. **METHODS**: Pinealectomy, luminol-dependent chemiluminescence (CL), hypothalamic dinoprostone radioimmunoassay, and intrahypothalamic injection of Mel were done in rats kept under light : dark 12 h : 12 h. **RESULTS**: CL value of PM∅ was decreased and hypothalamic dinoprostone content was elevated after pinealectomy, which were restored by Mel (10 $\mu g\ kg^{-1}\ d^{-1}$ ip at 16 : 00 for 7 d). The same treatment of Mel increased CL value of PM∅ and depressed hypothalamic dinoprostone production in intact rats. CL value of PM∅ showed negative relation to hypothalamic dinoprostone with $r = -0.78$ ($P < 0.01$). Intrahypothalamic injection of Mel (2 μg) enhanced CL value of PM∅ in normal and pinealectomized rats. **CONCLUSION**: The hypothalamus is a main site of pineal Mel action upon PM∅ function.

KEY WORDS pineal body; melatonin; peritoneal macrophages; chemiluminescence; hypothalamus; dinoprostone

The pineal body, as a neuroendocrine transducer by translating the most basic environmental information into signals that modulates neuroendocrine mechanisms[1,2], has been postulated to be involved in the functional connection between the immune and central nervous systems[3]. The immunoregulatory effects of melatonin (Mel), the major pineal hormone, were widely demonstrated by immuno-enhancing and oncostatic activity in animals. They play a modulatory role on inflammatory and immune responses[4-6]. However, the sites of Mel action are less known. The brain is the primary locus where Mel acts, and high affinity binding sites for Mel are found in several brain regions, particularly the hypothalamus[7,8]. Therefore, the present study was to investigate whether the hypothalamus was a major site for Mel action on macrophage functions.

Received 1994-06-09 Accepted 1995-04-17

MATERIALS AND METHODS

Rats Forty-five Sprague-Dawley rats (♂, 3—4 months old, 234 ± 46 g) were provided by Animal Center of Anhui Medical University. Rats were kept under light from 6:00 to 18:00 daily at 22±2 ℃ with free access and tap water.

Reagents and chemicals Mel, purchased from Sigma Chemical Co, was dissolved in 100 % ethanol and then diluted with normal saline to a final concentration of 2 % ethanol. Luminol (Sigma) was dissolved in triethylamine and phosphate-buffered saline (PBS) to a concentration of 2 g L^{-1}. Zymosan (Sigma) was suspended in PBS, boiled for 15 min, and then resuspended in DMEM (Gibco) at 50 g L^{-1}. Opsonized zymosan was made by incubating boiled zymosan with equal volume of fresh autologous serum at 37 ℃ for 30 min and then resuspending in PBS. Dinoprostone radioimmunoassay kit was obtained from the Chinese Academy of Medical Sciences, Beijing.

Pinealectomy (PE) The operation was doner[9]. After 7 d, rats were injected ip with Mel 10 $\mu g\ kg^{-1}\ d^{-1}$ or vehicle for 7 d.

Chemiluminescence (CL) Rat peritoneal macrophage (PM∅) suspensions were prepared at a

concentration of 2×10^9 L^{-1}. After 500 μL of PMØ suspensions were incubated at 37 ℃ for 20 min, they were placed in the measuring chamber of a SHG-1 bio-or CL analyzer (Shanghai), 100 μL of freshly prepared luminol 0.1 mmol L^{-1} and 100 μL of zymosan particles 5 g L^{-1} were added. CL was continuously recorded by a computer and a printer connected to the CL machine[10].

Dinoprostone radioimmunoassay In 30 mg of hypothalamus[6].

Intrahypothalamic injection of Mel Rats were anesthetized with 10 % chloral hydrate (300 mg kg^{-1} ip) and stereotaxically implated (P 1.0, L 0.1, H 8.0)[11] with a chronic cannula guide tube fabricated from 22-gauge thin-wall stainless steel hypodermic needle and contained an indwelling stylus made from 27-gauge stainless steel wire. The guide tube was anchored on the skull. Eight days later, rats received intrahypothalamic injection of Mel at 16:00 for 7 d. For injection, a 10-μL Hamilton syringe was inserted into the guide tube so that its tip extended 0.5 mm into the brain tissue. Before injection, the syringe was washed in 70 % ethanol. Mel 2 μg was injected in 1 min and the syringe was remained for another 1 min. 1 μL of 1 % ethanol in saline (vehicle) was given for control injection.

A total of 20 implant operations were performed, and all rats recovered well. The hypothalamic area was frozen in liquid nitrogen, sectioned with a cryostat, fixed in cold acetone, and stained with crysyl violet and eosin. By reference to an atlas[11], in all but 2 rats the injection cannulae were present in the vicinity of the suprachiasmatic and anterior hypothalamic area. The 2 rats showing injection sites in the thalamus were discarded.

Statistics Correlation coefficient was calculated using Casio fx-4200 P calculator. Statistical analyses were carried out using *t* tests.

RESULTS

Mel 10 μg kg^{-1} counteracted the decreased PMØ CL and increased hypothalamic dinoprostone production induced by PE. The same treatment of Mel increased PMØ CL and the inhibited dinoprostone production in intact rats (Tab 1). There was an inverse relationship between PMØ CL and hypothalamic dinoprostone production ($\hat{Y} = 5580 - 80X$, $r = -0.78$, $P < 0.01$).

Tab 1. Effects of pinealectomy (PE) and ip melatonin (Mel) at 16:00 on dinoprostone production in hypothalamus and chemiluminescence (CL) of peritoneal macrophages in rats. $n = 5$, $\bar{x} \pm s$. $^cP < 0.01$ *vs* control, $^fP < 0.01$ *vs* PE.

	Dose/ μg kg^{-1} d^{-1} × 7 d	CL/ cpm	Dinoprostone/ ng g^{-1}
Control	—	3 039±506	20±4
Mel	10	6 413±1075^c	11.2±2.1^c
Sham-PE	—	2 988±330	19.3±2.4
PE	—	1 166±215^c	58±11^c
PE+Mel	10	3 428±204^f	23.4±2.2^f

Intrahypothalamic injection of Mel (2 μg) increased PMØ CL in intact rats compared to those given vehicle injections. It also restored the decreased PMØ CL in pinealectomized rats (Tab 2).

Tab 2. Effects of intrahypothalamic injection of Mel 2 μg for 7 d on CL of peritoneal macrophages in normal and pinealectomized (PE) rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control, $^fP < 0.01$ *vs* PE.

	n	Dose/ μg d^{-1} × 7 d	CL/ 10^{-3} × cpm
Vehicle	4	—	1.86±0.24
Mel	5	2	3.0±0.6^c
PE	5	—	0.72±0.21^c
PE+Mel	4	2	1.57±0.27^f

DISCUSSION

Our present study demonstrated that dinoprostone content in the hypothalamus was increased by PE, which could be reversed by ip Mel. Mel also decreased dinoprostone production in the hypothalamus in intact rats. Hypothalamic prostaglandins play an important role in regulating pituitary function, *ie*, microinjection of prostaglandins in the

hypothalamus can effectively stimulate the release of LH, FSH, PRL, GH, and ACTH, etc[12]. So pineal Mel might modulate pituitary function via hypothalamic prostaglandins, thus affecting neuro-endocrino-immune network. Our result showed an inverse relationship between PMØ CL and hypothalamic dinoprostone production, suggesting that pineal Mel might regulate macrophage functions via hypothalamus. Intrahypothalamic injection of Mel enhanced PMØ CL in intact and pinealectomized rats. These observations indicated that the hypothalamus was the main site of pineal Mel action on macrophage functions.

REFERENCES

1 Xu SY, Xu DJ, Wei W. Some advances in pineal body research. Chin J Clin Pharmacol 1991; 7: 13—9.

2 Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr Rev 1991; 12: 151—80.

3 Maestroni GJM. The immunoneuroendocrine role of melatonin. J Pineal Res 1993; 14: 1—10.

4 Ding CH, Wei W, Xu SY. Pineal melatonin and inflammatory-immuno-regulation. Chin Pharmacol Bull 1994; 10: 84—8.

5 Wei W, Liang JS, Shen YX, Ding CH, Xu SY. Regulatory effects of pineal gland and melatonin on inflammatory and immune responses. In: Maestroni GJM, Conti A, Reiter RJ, editors. Advances in pineal research: 7. London: John Libbey, 1994: 131—6.

6 Ding CH, Wei W, Xu SY. Effects of pineal body and melatonin on lymphocyte proliferation and dinoprostone production in rat spleen. Acta Pharmacol Sin 1995; 16: 54—7.

7 Stankov B, Fraschini F, Reiter RJ. Melatonin binding sites in the central nervous system. Brain Res Rev 1991; 16: 245—56.

8 Yin SW, Zhang JZ. Effect of melatonin on brain function. Prog Physiol Sci 1987; 18: 251—5.

9 Kuszak J, Rodin M. A new technique of pinealectomy for adult rats. Experientia 1977; 33: 283—4.

10 Wang TP, Li Q, Shi XG, Ding CH, Yan CZ, Zhang XZ *et al*. Oxygen free radical changes of polymorphonuclear leukocytes in coronary heart disease of different types. Beijing Med J 1993; 15: 206—8.

11 Bao XM, Shu SY. The stereotaxic atlas of the rat brain. Beijing: People's Health Press 1991; 31—3.

12 Ojeda SR, Naor Z, McCann SM. Prostaglandin E levels in hypothalamus, median eminence and anterior pituitary of rats of both sexes. Brain Res 1978; 149: 274—7.

435-437 13

松果体和褪黑激素通过下丘脑影响大鼠腹腔巨噬细胞化学发光

R965.2

魏 伟, 丁长海, 徐叔云
(安徽医科大学临床药理研究所, 合肥230032, 中国)

目的: 研究松果体和褪黑激素(Mel)是否通过下丘脑影响腹腔巨噬细胞功能. **方法**: 松果体切除术; 腹腔巨噬细胞化学发光测定; 下丘脑地诺前列酮放射免疫测定; 下丘脑注射Mel. **结果**: 松果体切除后腹腔巨噬细胞化学发光值降低, 下丘脑地诺前列酮含量升高, 16:00 ip Mel (10 $\mu g\ kg^{-1}\ d^{-1} \times 7$ d)可使其恢复, 并升高正常大鼠腹腔巨噬细胞化学发光值, 降低其下丘脑地诺前列酮含量. 腹腔巨噬细胞化学发光值与下丘脑地诺前列酮含量的变化存在负相关(相关系数 $r = -0.78$, $P < 0.01$). 于下丘脑注射Mel 2 μg, 能提高正常大鼠和松果体切除大鼠腹腔巨噬细胞化学发光值. **结论**: 下丘脑是松果体Mel影响腹腔巨噬细胞功能的主要作用部位之一.

关键词 松果体; 褪黑激素; 腹腔巨噬细胞; 化学发光; 下丘脑; 地诺前列酮

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Sep; 16 (5): 438—440

Hippocampal quinolinic acid concentrations in epileptogenesis in rats

GUO Qing[1], KUANG Pei-Gen (*Neurotransmitter Laboratory, Department of Neurology, PLA General Hospital, Beijing 100853, China*)

AIM: To study the changes of hippocampal quinolinic acid (QA) concentrations during acute and chronic seizures induced by ip injection of kainic acid (KA, 12 mg kg^{-1}) in rats. **METHODS**: The extraction and measurement of QA in the hippocampus were performed using a gas chromatography-mass spectrometry method. **RESULTS**: When acute seizures were fully established 3 h after KA injection, no significant changes of hippocampal QA were found. During chronic seizures observed on d 30 after KA injection, there was even a 55 ± 8 % significant decrease. When neither acute nor chronic seizures were detectable but astroglial proliferation in the hippocampus and secondary neuronal degeneration in extrahippocampal regions became gradually prominent 2 d and 7 d after KA injection, there were 56 ± 13 % and 156 ± 13 % dramatic increases of hippocampal QA concentrations, respectively. **CONCLUSION**: The increase of hippocampal QA hardly plays any key role in the initiation of KA-induced seizures but may contribute to astroglial proliferation and neuronal degeneration by activation of *N*-methyl-*D*-aspartate receptors.

KEY WORDS epilepsy; hippocampus; quinolinic acid; kainic acid; mass fragmentography

Since quinolinic acid (QA) in the CNS is the most potent member of kynurenine metabolic pathway in causing convulsions and cell death, QA is worthy of serious consideration as a causative factor in convulsive disorders[1-3]. Our preliminary work found no increase, but a decrease, of QA in cerebrospinal fluid (CSF) of epileptic patients compared with controls[4]. This decrease was neither the effect of antiepileptic medication nor the effect of the causes of seizures[4]. The result did not exclude the possibility that early increases in brain QA were involved in pathogenesis of epilepsy, since all these patients had had recurrent seizures for 6 ± 8 a[4]. In the present study, kainic acid (KA) model of temporal lobe epilepsy[5, 6] was used to analyze the hippocampal QA concentrations at different time points after the establishment of acute and chronic seizures in rats.

MATERIALS AND METHODS

Wistar rats (♂, 50 for KA-treated and 12 for control group) weighing $196 \pm s$ 16 g were decapited at different time points after ip injection of KA (12 mg kg^{-1})[5, 6]: h 3 ($n=12$), d 2 ($n=10$), d 7 ($n=15$), and d 30 ($n=13$). Control rats ($n=12$, 3 for each time points) were injected with the solvent phosphate buffer solution (PBS, pH 7.4) alone. After the injection, rat behavior was monitored for 30 d.

The extraction and measurement of QA in the hippocampus were performed using a gas chromatography-mass spectrometry method previously described[7] with some modifications. In brief, the hippocampus was homogenized in 2 mL 80 % ethanol containing 100 μL NaOH 0.5 mol L^{-1} and 2,4-pyridine dicarboxylic acid (2,4-PDA) 2 nmol L^{-1} as internal standard. The hippocampal QA and 2,4-PDA were derivatized to hexafluoroisopropanol (HFIP) ester, which was then dissolved in 10 μL acetone. Two μL of this solution was injected into the gas chromatography-mass spec-

[1] Now in *Department of Neurobiology, Institute of Basic Medical Sciences, Academy of Military Medical Sciences, P O Box 130(3), Beijing 100850, China.*

Received 1994-05-03 Accepted 1995-03-03

trometer (GC-MS 2000). The following GC were used: sylanized glass column (2.5 m × 0.2 mm) containing 5 % OV-17 on Chromosorb, 100—200 mesh; helium flow rate: 15 mL min^{-1}; oven temperature 145 ℃; flash heater temperature 190 C. MS conditions were: separator temperature 240 C; electron energy 60 eV; acceleration voltage 3.5 kV. Under such ionization conditions, the predominant ion had mass/charge (m/z) of 272, therefore the content of QA was obtained by comparing the height of its peak with that of its internal standard at m/z 272. Data were expressed as $\bar{x}\pm s$ and statistically analyzed using t test.

RESULTS

The behavioral seizures within 1 d after KA injection were called acute seizures, characterized by "wet dog shakes", forepaws tremor, loss of posture control, and generalized seizures. Three hours after KA injection, the rats exhibited a fully developed status epilepticus. We therefore selected this time point to assess the possible changes of hippocampal QA concentrations which may occur in conjunction with acute seizures. However, no such early changes were detected (Tab 1).

Tab 1. Hippocampal quinolinic acid (QA) concentrations (nmol/g wet tissue) at different time points after ip kainic acid 12 mg kg^{-1}. Number of rats in parentheses. $^bP<0.05$, $^cP<0.01$ *vs* control.

Time after KA	Control	KA
3 h	0.91±0.20 (3)	0.91±0.32 (12)
2 d	0.82±0.26 (3)	1.28±0.31 (10)^b
7 d	0.80±0.13 (3)	2.05±0.43 (15)^c
30 d	0.93±0.25 (3)	0.42±0.15 (13)^b

All the rats showed a relatively "silent" period with no seizure behavior 2—7 d after KA. But compared with control, QA concentrations in the hippocampus increased 56± 13 % and 156±13 % on d 2 and d 7, respectively.

The recurrent seizures occurred 8—30 d after KA treatment were called chronic seizures, characterized by generalized seizures. A 55±8 % decrease in QA concentrations in the hippocampus was detected 30 d after KA treatment.

DISCUSSION

The time course of changes of hippocampal QA concentrations was determined in the present study to examine if there was any increase of brain QA which might be involved in the initiation of KA-induced acute and/or chronic behavioral seizures. When acute seizures were well established 3 h after KA treatment, no significant change in hippocampal QA concentrations was detected. Hippocampal QA was even decreased during chronic seizures 30 d after KA injection. The increases of hippocampal QA concentrations were observed 2—7 d after KA administration, which corresponded temporally with neither acute nor chronic seizures, but with a relatively "silent period" for behavioral responses. These results indicated that the activation of NMDA receptors by QA hardly played a key role in the initiation of KA-induced acute and chronic seizures. This conclusion is consistent with the observation that NMDA antagonists failed to block the initiation of KA-induced epileptic burst discharges in hippocampal CA3 neurons[8]. Besides, the decrease in hippocampal QA concentrations during KA-induced chronic seizures is consistent with our previous clinical observations showing that CSF QA concentrations in chronic epileptic patients also decreased markedly[4].

KA causes neuron loss and astroglial proliferation in both hippocampal and some distinct extrahippocampal (distant) regions[5]. These pathological changes were particularly pronounced between 2—7 d after KA injec-