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Binding sites of mu receptor increased when acupuncture analgesia was enhanced by droperidol: an autoradiographic study[1]

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AIM: To study if μ receptor participates in the process of potentiation of droperidol (Dro) on acupuncture analgesia (AA). **METHODS**: Autoradiographic technic was used. Ohmefentanyl, a highly selective ligand of μ receptors, was used in radio-receptor binding assay in Sprague-Dawley rat brain sections. **RESULTS**: The binding sites of [β-^{3}H, *p-benzoyl*-^{3}H]ohmefentanyl were increased greatly in many nuclei of rat brain after AA, and were further increased when AA was enhanced by Dro. Higher increase was seen in caudate nucleus, accumbens, periaqueductal gray (PAG), interpeduncular nucleus, amygdala ($P<0.01$ *vs* rats treated with electroacupuncture alone); moderate increase was noted in thalamus, lateral area of hypothalamus, spinal dorsal horn ($P<0.01$ or 0.05); slight increase appeared in septum, preoptic area, hippocampus, substantia nigra ($P<0.05$). **CONCLUSION**: Mu opioid receptors mediated the Dro-induced enhancement of AA.

KEY WORDS mu opioid receptors; acupuncture analgesia; droperidol; autoradiography; ohmefentanyl; central nervous system

Antagonists of dopamine receptors potentiate acupuncture analgesia (AA)[1,2]. Our previous study showed that the density of opioid receptors was increased when haloperidol (dopamine receptor antagonist) potentiated AA[3]. However, which type involved still remains unclear. Since the μ receptor plays an important role in pain-modulation and mediating antinociception in central nervous system[4], this study was designed to measure the binding sites of μ receptor by using receptor-binding autoradiography and computer-assisted image analysis system to determine the change trend of μ receptor when AA was enhanced by droperidol.

MATERIALS AND METHODS

Measurement of pain threshold Sprague-Dawley rats (♂, $n=19$, $210\pm s$ 40 g, supplied by the Department of Experimental Animals, Chinese Academy of Sciences) were divided into 4 groups: A) Normal saline (NS, 2 mL, ip, $n=4$), B) NS + electroacupuncture (EA) ($n=5$), C) droperidol (Dro, 1.25 mg kg^{-1}, ip, $n=5$) and D) Dro+EA ($n=5$). The treatment of the rats conformed to the guidelines of International Association for Study of Pain[5]. Pain threshold was measured with Model WQ-9E Pain Threshold Meter (Beijing). The basic pain threshold of each rat was tested thrice, of which the mean value in normal rats ranged from 0.1 to 0.2 mA. The pain threshold was measured successively after medication at the interval of 10 min.

EA EA was applied unilaterally at "Zu-San-Li" (St 36, between the muscle anterior tibialis and muscle extensor digitorum longus) and "Kun-Lun" (UB60, between the tip of the external malleolus and tendo calcaneus) points (the needles were inserted 5 mm) on right side with Model G6805 EA Apparatus (Shanghai) at the 10th min following drug or NS injection and kept on for 20 min.

Tissue preparation The rats which exhibited the

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potentiating effect of the Dro on AA were selected. After the measurement of pain threshold, the rats were decapitated and the brains and lumbar spinal cords (L3-5) were sectioned coronally with SLEE cryostate (Germany) at −18 ℃. The sections (20 μm) were thaw-mounted onto gelatin-coated slides, stored at −20 ℃ for 24 h prior to incubation.

Radioligand binding and autoradiography The brain sections were incubated with [β-^{3}H, *p-benzoyl*-^{3}H] ohmefentanyl (3.6 nmol L^{-1}, 2.07 TBq mol^{-1}, Shanghai Institute of Materia Medica, Chinese Academy of Sciences) in Tris-HCl buffer (50 mmol L^{-1}) for 45 min at 25 ℃ to assess total binding. Adjacent sections were incubated in a solution containing radioligand in the presence of an 1000-fold excess of ohmefentanyl (3.6 μmol L^{-1}) to assess nonspecific binding. After washing and drying, the sections were exposed with tritium sensitive Hyperfilm (Amersham) for 5 wk at 4 ℃. Quantitation of the autoradiograms was achieved by averaging 3 or more readings from each quantitated nucleus using an image processing and analysis system (FG-100-AT, Imaging Technology Inc, and TV-Camera, RCA Inc, USA). Optical densities were converted to kBq/g protein by linear regression of concurrently exposed standards utilizing a double reciprocal plot. Brain structures were identified by reference to the rat atlas[5]. Statistical analysis of binding levels in various brain regions was managed with group *t*-test of 2 samples.

RESULTS

Enhancement of AA by droperidol The pain threshold was stable in NS group, but increased ($P<0.05$) in NS+EA or Dro group, the pain threshold showed a more increase ($P<0.01$) when Dro was combined with EA. The maximal increase of pain threshold appeared at 20 min after EA (just before the killing). These results indicate that the effect of AA was enhanced by Dro (Tab 1).

Increase of bindig sites of μ receptor Rats were decapitated 20 min after EA (when maximal increase of pain threshold was shown).

The binding sites of μ receptors were slight in the brain sections from control rats, and increased in most pain/analgesia-related nuclei after EA as well as when Dro was used alone in some nuclei, such as caudate nucleus, accumbens, septal nucleus, amygdala, PAG, areas related to dopaminergic system. When EA was combined with Dro, the binding sites appeared a further elevation in various brain regions than those from rats treated with EA or Dro alone. The increases of [β-^{3}H, *p-benzoyl*-^{3}H]ohmefentanyl binding in rats treated with EA + Dro were generally > 60 %, in some cases, even to 80 %; while in rat treated with EA alone, the increases in most nuclei were <40 % (Tab 2, Fig 1, Plate 1).

Tab 1. Net increase of pain threshold (μA) of rats ($\bar{x}\pm s$). $^bP<0.05$, $^cP<0.01$ *vs* NS group; $^eP<0.05$, $^fP<0.01$ *vs* NS+EA group.

Time after treatment	NS ($n=4$)	NS+EA ($n=5$)	Dro ($n=5$)	Dro+EA ($n=5$)
10 min	11±5	50±7	40±6	60±10
20 min	12±8	80±18^c	60±12	200±16^f
30 min	14±7	140±17^c	120±20^b	300±18^f

Higher increases of binding sites of μ receptors (>70 %) after EA+Dro were found in caudate nucleus, accumbens, medial centromedial, rhomboid nuclei of thalamus, medial area of hypothalamus, amygdala, interpeduncular nucleus, PAG, and superior colliculi. Moderate increases (60 %− 70 %) were noted in dorsal, centrolateral, pericentral and reticular nuclei of thalamus, lateral area of hypothalamus, and spinal dorsal horn. Slight but still significant increases (<60 %) were seen in preoptic area, hippocampus, septum, habenular, and substantia nigra.

DISCUSSION

The results in present study indicate that the potentiating effect of Dro on AA involves in promotion of the function of opioidergic

Tab 2. Bindings of [β-^{3}H, p-*benzoyl*-^{3}H]ohmefentany (kBq/g tissue) in rat brain in various groups of treatment. $n=4$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* NS; $^eP<0.05$, $^fP<0.01$ *vs* NS+EA; $^hP<0.05$, $^iP<0.01$ *vs* Dro.

	NS	NS+EA	Dro	Dro+EA
Telencephalon				
Caudate	440±90	596±92^c	494±94^b	782±112^{fi}
Accumbens	492±65	625±122^c	540±102^b	840±127^{fh}
Septum	171±28	214±20	191±27	253±21^{eh}
Preoptic area	122±13	151±39^b	127±16	188±46^{eh}
Diencephalon				
Hippocampus	167±17	198±33	168±13	249±20^{eh}
Habenular N	545±92	756±112^c	645±97^b	855±150^{eh}
Thalamus				
Medial dor N	245±15	344±29^c	302±30^b	423±35^{ei}
Later dor N	124±14	200±18^c	125±17	210±27^i
Centromed N	424±70	515±120^c	405±74	724±133^{fi}
Centrolat N	307±28	422±27^c	368±34^b	493±60^{ei}
Pericentr N	357±37	473±46^c	403±41^b	581±31^{fi}
Ventrolat N	135±11	184±14^b	148±14	217±28^{eh}
Ventromed N	297±27	379±58^c	319±23	478±37^{fi}
Rhomboid N	396±34	518±26^c	472±57^b	685±65^{fi}
Reticular N	245±23	386±37^b	228±16	426±34^{ei}
Amygdaloid N	305±57	392±77^c	337±54^b	545±75^{fi}
Hypothalamus				
Medial area	203±55	301±71^c	244±47^b	359±75^{ei}
Lateral area	152±42	172±53^b	136±33	245±72^{ei}
Mesencephalon				
Interpedun N	703±104	1 215±63^c	1 125±88^c	1 326±153^{fi}
Subs nigra	380±82	575±101^b	475±70	744±122^{eh}
PAG	475±74	724±82^b	563±87^b	947±102^{fi}
Sup colli	442±10	753±12^b	534±14^b	864±10^{ei}
Spinal cord				
Dorsal horn	202±52	270±81^b	240±72	525±112^{di}

system. The activity of dopaminergic system is one of the unfavorable factors to AA[6], which inhibits endogenous opioid peptide. When Dro was used to block the activity of dopaminergic system, the unfavorable factors to opioidergic system was diminished and the function of opiate system was promoted, showing a higher increase in μ receptor binding sites. In this way, AA was potentiated. However, it's difficult to distinguish μ_1 from μ_2, because ohmefentanyl can bind to the both. Most nuclei in which μ receptors were further increased following Dro enhancing AA are related to dopaminergic system. Some are areas containing dopaminergic neurons, some are areas accepting the dopaminergic projections, such as interpeduncular nucleus, habenular nucleus, caudate putamen, accumbens, amygdala, PAG, etc. These structures are very important in antinociception and densely distributed with mu-receptors[7]. However, the activity of dopaminergic system in these nuclei may partly inhibit the function of opioidergic system. When the activity of dopamine in these nuclei was blocked by Dro, EA was enhanced via diminishing the inhibition of opioidergic system. The increase of μ receptors suggest that the potentiating effect of Dro on AA is mediated, at least in part, by this type of opioid receptor.

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氟哌利多加强针刺镇痛时大鼠脑内 μ 受体结合位点增加：放射自显影研究[1]

R965.2

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目的：观察 μ 受体在氟哌利多加强针刺镇痛过程中的变化. **方法**：运用放射自显影技术，以 μ 受体的高选择性配基羟甲芬太尼在大鼠脑片上作放射受体结合分析. **结果**：针刺使^{3}H-羟甲芬太尼与 mu 受体的结合在大鼠脑内许多核团明显增加；当氟哌利多增强针刺镇痛时，这一结合进一步增加. 增加程度较大的核团有尾核，伏核，视前区，中脑导水管周围灰质（$P<0.01$）；中等程度的增加可见于丘脑，下丘脑外侧区，脊髓背角等（$P<0.01$ 或 0.01）；增加程度较小者有隔核，视前区，黑质（$P<0.05$）. **结论**：μ 受体介导了氟哌利多对针刺镇痛的加强作用.

关键词 μ 阿片受体；针刺镇痛；氟哌利多；放射自显影；羟甲芬太尼；中枢神经系统