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ANTINOCICEPTIVE EFFECT OF SPINAL AND SYSTEMIC PHYSOSTIGMINE: EARLY VERSUS LATE POSTOPERATIVE PERIOD

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Introduction. *The purpose of the present study was to compare the antinociceptive effect of spinally administered cholinesterase inhibitor physostigmine during the acute and late phases of postoperative period in rats. We also evaluated the effect of the surgery on the antinociception induced by a systemic physostigmine, since this effect is partially realized via the spinal antinociceptive mechanisms, and subsequently may be under the influence of a spinal cholinergic tone.*

Material and methods. *Under general a small incision was made in the atlanto-occipital membrane. A PE 10 catheter was introduced to a length of 10 cm caudal with its internal tip located at the level of the lumbar enlargement. The second catheter was inserted into the jugular vein. The catheter was then directed under the skin towards the dorsal surface of the neck. Both catheters were secured to the muscles at the back of the neck, the muscles and the skin were sutured and anesthesia was discontinued. Within one hour after the completion of the surgery animals were completely recovered. Nociception was evaluated in the «plantar stimulation» test. Changes in nociception were determined by the changes in response latencies to noxious stimulation of the hind paw. In order to minimize tissue injury, a cut-off time of 15 sec was imposed.*

Results. *Intravenous administration of physostigmine 1–4 hours after the surgery in the doses of 50 or 100 μg/kg resulted in a dose-dependent increase in the response latencies. Statistical analysis also demonstrated that the effect of 100 μg/kg of IV physostigmine was significantly more pronounced during the early postoperative period if compared to the effect of the same dose injected 3–5 days after the surgery.*

Conclusion. *The results of the present and previous similar studies are of a significant clinical value since immediate postoperative pain might be an indication for the future use of cholinesterase inhibitors as analgesic agents.*

Key words: *nociception, physostigmine, spinal cord, analgesia*

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INTRODUCTION

Spinal administration of cholinomimetics and cholinesterase inhibitors results in an antinociceptive effect [1]. Cholinesterase inhibitors produce antinociception in such animal species as rats, but fail to do so in sheep [2, 3]. Since the effect of cholinesterase inhibitors depends on the concentration of ACh, these substances should be able to produce antinociceptive effect of different magnitude depending on the degree of spinal cholinergic activity. Spontaneous spinal cholinergic tone was shown to be present in rats [4]. Sheep probably lack spontaneous spinal cholinergic tone, however they were able to demonstrate antinociception in response to spinal neostigmine administration during an early postoperative period [5]. The increased cerebrospinal fluid (CSF) level of ACh during the stimulation of nociceptive primary afferents [6] may account for this antinociceptive effect.

It was suggested that during the early phase of a postoperative period central endogenous mechanisms involving a spinal cholinergic link are activated in sheep by nociceptive stimulation, which increases the CSF level of ACh and consequently the effect of cholinesterase inhibitor neostigmine [5].

It is still unknown whether this effect is unique for sheep or it can be observed also in other animal species, including those that have spontaneous spinal cholinergic tone (such as rats).

The purpose of the present study was to compare the antinociceptive effect of spinally administered cholinesterase inhibitor physostigmine during the acute and late phases of postoperative period in rats. We also evaluated the effect of the surgery on the antinociception induced by a systemic physostigmine, since this effect is partially realized via the spinal antinociceptive mechanisms, and subsequently may be under the influence of a spinal cholinergic tone.

MATERIAL AND METHODS

Experiments were approved by the Institutional Animal Care and Use Committee of the University of Southern California. Male Sprague Dawley rats weighing 300–350 g at the time of surgery (intrathecal and intravenous catheterization) were used in these experiments. They were housed individually in a room maintained at 23 °C having a 12 hr light-dark cycle. Food and water were available ad libitum. Tests were performed during the light-on phase. For intrathecal (IT) injections we used a subarachnoid catheter inserted according to a technique previously developed by Yaksh and Rudy [7]. For intravenous (IV) injections we used a catheter inserted into the jugular vein.

Under general anesthesia (O_2, N_2O, and halothane), after depilating the skin of the neck and a midline skin incision over the spinous process of the cervical vertebrae, a small incision was made in the atlanto-occipital membrane. A PE 10 catheter was introduced to a length of 10 cm caudal with its internal tip located at the level of the lumbar enlargement. The second skin incision was made on the ventral surface of the neck, and the PE 50 catheter was inserted into the jugular vein. The catheter was then directed under the skin towards the dorsal surface of the neck. Both catheters were secured to the muscles at the back of the neck, the muscles and the skin were sutured and anesthesia was discontinued. Within one hour after the completion of the surgery animals were completely recovered. The animals demonstrating any signs of neurological deficit or abnormal behavior after the surgery were excluded from the experiment. Immediately after the surgery the animals were randomly assigned to one of the two experimental groups. The first group of animals underwent the experimental procedure 1–4 hours after the surgery; the second group underwent the same experiment 3–5 days after the surgery. Nociception was evaluated in the «plantar stimulation» test [8]. Changes in nociception were determined by the changes in response latencies to noxious stimulation of the hind paw. In order to minimize tissue injury, a cut-off time of 15 sec was imposed.

Baseline response latency was defined as the mean of three determinations performed at 5-min intervals before any drug injection. Following drug administration, response latencies were measured during a period of two hours, and the time of peak antinociceptive effect and the duration of this effect were determined.

All experimental substances were dissolved in 0.9 NaCl and injected either IT in a volume of 10 μl, flushed in with 10 μl of normal saline, or IV in a volume of 1 ml/kg.

At the end of the experiment, all rats with spinal catheters received an IT injection of 10 μl of 2 % lidocaine. Data from rats that did not develop motor paralysis within 3 min were excluded. The nociceptive response of each rat was converted to percent of maximal possible effect (% MPE):

$$\% \text{ MPE} = \frac{(\text{postdrug response} - \text{predrug response})}{(\text{cut-off time} - \text{predrug response})} \cdot 100,$$

where: postdrug response = the longest response latency observed after drug administration; predrug response = the mean of three determinations made before drug administration; cut-off time = 15 sec.

Comparisons between groups of animals were carried out with a one-way ANOVA. Paired comparisons were performed using Student-Newman-Keuls multiple comparison test. A P value <0,05 was considered significant.

RESULTS

Physostigmine 10 or 20 μg IT administered 1–4 hours after the surgery increased the latencies of nociceptive response in a dose-dependent manner (Fig. 1). The % MPE was equal to 26,7±4.7 or 43,55±3,8, respectively. The antinociceptive effect reached its maximum 10 min and returned to the baseline 45 or 60 min after the administration of 10 or 20 μg of physostigmine, respectively. The difference between the effects of two doses was statistically significant (Fig. 2). Similar significant difference was also noted when AUC for both doses were compared (Fig.2).

When the same doses of physostigmine were administered 3–5 days after the surgery, the latencies of nociceptive responses as well as the duration of the antinociceptive effect significantly decreased (Fig. 1). The % MPE was equal to 17,2±1,9 or 21,6±4,6 for 10 or 20 μg, respectively (Fig. 2). The difference between the effects of two doses expressed either as % MPE or AUC, was not statistically significant (Fig.2). Statistical analyses of the data also revealed that the effect of 20 μg of IT physostigmine administered 1–4 hours after the surgery

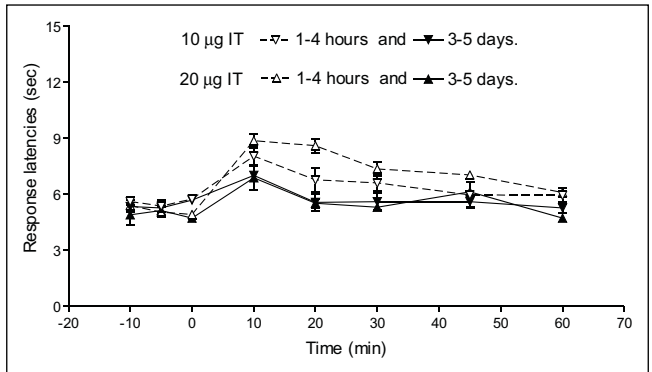

Fig. 1. Effect of physostigmine 10 or 20 μg IT on the latencies of nociceptive responses in rats 1–4 hours or 3–5 days after the surgery. All points represent the mean response latencies of 5–6 animals. «0 min» represents the time of the drug administration. Error bars denote SEM

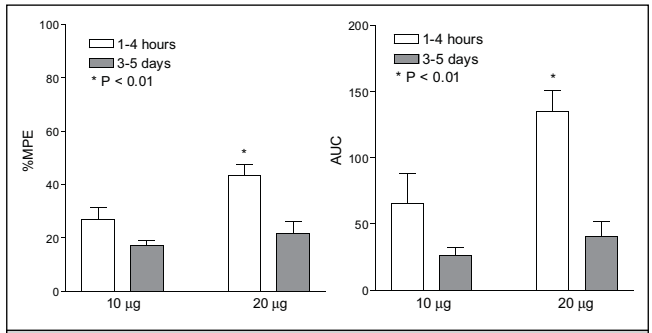

Fig. 2. Effect of physostigmine 10 or 20 μg IT on nociception in rats 1–4 hours or 3–5 days after the surgery. Vertical bars represent the mean % of maximal possible effect (% MPE) or the mean area under the curve (AUC) of 5–6 animals. Error bars denote SEM

was significantly greater than the effect of the same dose administered 3–5 days after the surgery.

Intravenous administration of physostigmine 1–4 hours after the surgery in the doses of 50 or 100 μg/kg resulted in a dose-dependent increase in the response latencies (Fig.3). The % MPE was 13,7±6,6 or 42,8±7,6 for 50 or 100 μg/kg, respectively (Fig.4). The difference between the effects of two doses was statistically significant. The difference was also statistically significant when the AUC were compared (Fig.4). When the same doses were injected 3–5 days after the surgery the animals demonstrated less pronounced increase in the response latencies (Fig. 3). The % MPE was equal to 11,9±0.8 or 3,9±0,9 for 50 or 100 μg/kg, respectively (Fig. 4). The difference between the effects was not statistically significant. A similar non-significant difference was observed when the AUC were calculated (Fig. 4). Statistical analysis also demonstrated that the effect of 100 μg/kg of IV physostigmine was significantly more pronounced during the early postoperative period if compared to the effect of the same dose injected 3–5 days after the surgery.

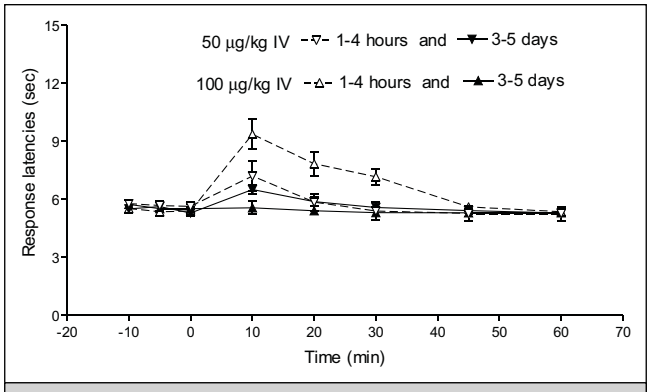

Fig. 3. Effect of physostigmine 50 or 100 μg/kg IV on the latencies of nociceptive response 1–4 hours or 3–5 days after the surgery. All points represent the mean response latencies of 6–7 animals. «0 min» represents the time of the drug administration. Error bars denote SEM

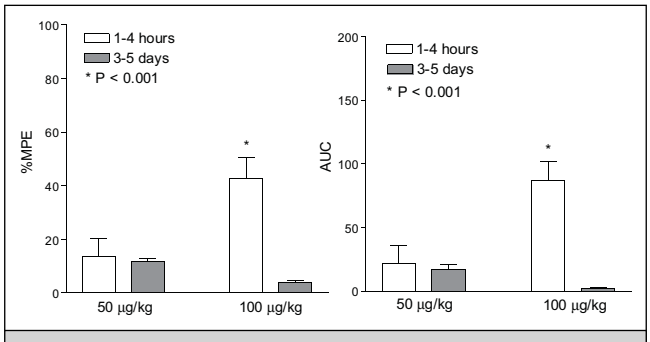

Fig. 4. Effect of physostigmine 50 or 100 μg/kg IV on nociception in rats 1–4 hours or 3– days after the surgery. Vertical bars represent the mean % of maximal possible effect (% PE) or the mean area under the curve (AUC) of 6– animals. Error bars denote SEM

In order to evaluate the role of spinal cholinergic mechanisms in the antinociceptive effect of systemically administered cholinesterase inhibitor, we performed an additional series of experiments with IV administered physostigmine and IT administered m-cholinergic blocker atropine. Both drugs were administered in the acute postoperative period, since during this period physostigmine demonstrated maximum of its antinociceptive effect. Atropine injected alone in a dose of 10 μg IT was not able to produce any changes in the latencies of nociceptive responses of experimental animals. There were also no changes observed in the nociceptive threshold when physostigmine 100 μg/kg IV was injected 20 min following atropine administration. The % MPE of the combination of atropine and physostigmine was significantly smaller than the % MPE of physostigmine alone and did not differ significantly from that of controls (Fig. 5).

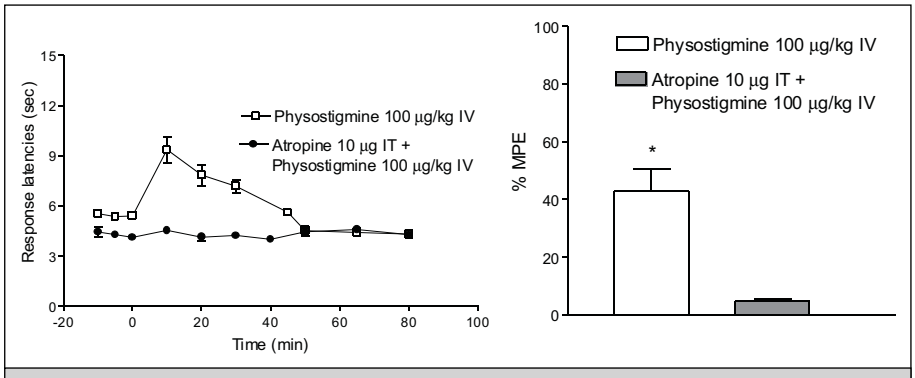

Fig. 5. Effect of atropine injected IT on the antinociceptive effect of IV physostigmine, expressed as the latencies of nociceptive response (left panel) or % of Maximal Possible Effect (right panel), in rats 1-hours after surgery. Physostigmine was injected in a dose of 100 μg/kg 20 min after the injection of atropine 10 μg. «0 min» represents the time of the administration of atropine or physostigmine (when injected alone). Error bars denote SEM

DISCUSSION

The present study demonstrated that IT or IV administration of cholinesterase inhibitor physostigmine immediately following the surgery in rats resulted in a more pronounced antinociceptive effect than administration of the same doses of the drug at least 3 days after the surgery. This suggested that the activity of spinal cholinergic systems and consequently the level of ACh were increased during the early postoperative period. The ability of spinal atropine to prevent the effect of systemic physostigmine suggests that cholinergic mechanisms activated during the acute postoperative period are located at the spinal rather than supraspinal level.

The results of this study are consistent with the previous data of Bouaziz et al. [5] who demonstrated that cholinesterase inhibitor neostigmine, ineffective in sheep 5 days after surgery, did cause antinociception in the acute postoperative period.

Based on our study and previous experiments we can suggest that early postoperative period develops a state which activates endogenous systems containing a spinal cholinergic link. This phenomenon can probably be observed in various animal species regardless of the level of spontaneous cholinergic activity.

We do not know exactly what might be a trigger for the increased activity of spinal cholinergic neurons.

It was hypothesized by Bouaziz et al. [5] that postoperative pain enhanced spinal cholinergic tone and, therefore, the effect of cholinesterase inhibitor. This hypothesis was confirmed by Eisenach et al. [6] who demonstrated that electrical stimulation of nociceptive primary afferents resulted in an increased level of ACh in the CSF.

Several lines of evidence support this hypothesis, although the mechanism of this effect is not clear.

A number of behavioral, electrophysiological and biochemical experiments have shown that a noxious stimulus applied to one part of the body was able to suppress the response to another, spatially remote, noxious stimulus [9–16]. Endogenous antinociceptive systems appeared to operate through propriospinal as well as supraspinal mechanisms. The latter is referred to as Diffuse Noxious Inhibitory Controls (DNIC) [17]. The transmitter which is released upon the activation of the descending limb of DNIC is probably serotonin [18], although there is an indication that norepinephrine also may be involved [19]. The transmitter in propriospinal systems is believed to be ACh [14]. It was suggested that descending NE neurons involved in DNIC might stimulate these systems and consequently the release of ACh [6]. However, it may be that neither NE nor DNIC are involved in the activation of the propriospinal cholinergic systems. Zhuo and Gebhart [14] demonstrated that noxious colorectal stimulation inhibited the thermally induced nociceptive reflex. This effect was reduced by an intrathecal pretreatment with methysergide or atropine, but not phentolamine, and transaction of dorsolateral funiculi did not alter the effect of cholinergic antagonist. In addition, a recent clinical study [20] suggests that acute postoperative pain might be a type of pain which is not able to induce DNIC.

Another trigger, which may increase the activity of endogenous antinociceptive systems, is stress associated with surgery. It has been shown in a number of studies that stressful environmental situations are capable of producing antinociception [21–24]. These phenomena are collectively termed Stress-Induced Analgesia (SIA). Antinociceptive responses elicited by various environmental events differ in their pharmacological profiles as well as morphological substrates. Some forms of SIA are believed to utilize supraspinal sites which mediate analgesia via descending pathways, others involve propriospinal pathways as well. Opioid and non-opioid forms of SIA were demonstrated, and cholinergic link is believed to be involved in both of them [25, 26]. The site of cholinergic link has not been adequately investigated. It was proposed to exist at the supraspinal rather than spinal level [27, 28], however this was demonstrated only in the model of footshock induced analgesia (FSIA).

Since surgery is a stressful event, we may assume that it activates endogenous mechanisms capable of inhibiting nociception. These mechanisms can engage cholinergic systems, which will result in an increased level of ACh and effect of cholinesterase inhibitor.

We can conclude that during the early postoperative period, nociceptive stimuli by themselves may trigger endogenous antinociceptive systems. Nociceptive stimuli originating from the surgical wound may induce SIA as well. Both events, by utilizing the same and/or different pathways, eventually result in activation of spinal cholinergic neurons with subsequent increase in the CSF level of ACh and effect of cholinesterase inhibitor.

CONCLUSION

The results of the present and previous similar studies are of a significant clinical value since immediate postoperative pain might be an indication for the future use of cholinesterase inhibitors as analgesic agents.

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АНТИНОЦИЦЕПТИВНЫЙ ЭФФЕКТ ФИЗОСТИГМИНА ПРИ СПИНАЛЬНОМ И СИСТЕМНОМ ВВЕДЕНИИ В РАННЕМ И ПОЗДНЕМ ПОСЛЕОПЕРАЦИОННОМ ПЕРИОДЕ

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РЕЗЮМЕ

Введение. Проводили сравнение антиноцицептивного эффекта ингибитора холинэстеразы физостигмина при спинальном введении в острой и поздней фазах послеоперационного периода у крыс, а также оценивали влияние операции на антиноцицепцию, вызванную системным введением физостигмина, так как этот эффект частично реализуется через спинальные антиноцицептивные механизмы и впоследствии может контролироваться состоянием холинергического тонуса.

Материал и методы. Под общей анестезией в атланто-затылочной оболочке был выполнен небольшой надрез. Катетер PE 10 вводили каудально в спинно-мозговой канал на длину 10 см, что соответствовало уровню поясничного расширения. Второй катетер был введен в яремную вену. Затем катетеры были направлены подкожно к дорзальной поверхности шеи. Оба катетера были прикреплены к мышцам в задней части шеи. В течение 1 ч после завершения операции состояние животных полностью восстанавливалось. Ноцицепцию оценивали в тесте «плантарной стимуляции». Изменения ноцицепции определялись изменениями латентности ответа на раздражение задней лапы. Чтобы свести к минимуму повреждение ткани, было введено время отсечки – 15 с.

Результаты. Внутривенное введение физостигмина через 1–4 ч после операции в дозах 50 или 100 мкг/кг приводило к дозозависимому увеличению латентного периода ответа. Согласно статистическому анализу, эффект 100 мкг/кг IV физостигмина был значительно более выраженным в раннем послеоперационном периоде по сравнению с эффектом той же дозы, введенной через 3–5 дней после операции.

Заключение. Результаты настоящих и предыдущих аналогичных исследований имеют важное клиническое значение, поскольку немедленная послеоперационная боль может быть показанием для применения ингибиторов холинэстеразы в качестве анальгетиков.

Ключевые слова: ноцицепция, физостигмин, спинной мозг, анальгезия