EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 7
543-548
Year: 2021

ANALGESIC INTERACTION BETWEEN INTRATHECALLY ADMINISTERED BUPIVACAINE AND EPIBATIDINE IN RATS

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Article Received on 18/05/2021 **Article Revised on 08/06/2021** **Article Accepted on 28/06/2021**

ABSTRACT

Background: We investigated the interaction between intrathecally administered bupivacaine and epibatidine, a nicotinic acetylcholine receptor agonist, on analgesic effects in acute thermal and formalin induced pain models of rats. **Methods:** Male Sprague-Dawley rats implanted with lumbar intrathecal catheters were given intrathecal combination of 1/2, 1/4, 1/8, and 1/16 50% effective doses (ED50s) of bupivacaine and epibatidine, then tail flick test or formalin test was performed. Isobolographic analysis was done using ED50s, and total fractional dose values were calculated. Behavioral side effects were checked. **Results:** In the tail flick test, ED50 of the combination was not obtained. Dose dependent decreases of number of flinch response were observed in the formalin test. The ED50s of both phase 1 and 2 of the formalin test were significantly lower than the theoretical additive values. Side effects were not observed in this study. **Conclusions:** Intrathecal bupivacaine and epibatidine had antagonistic effects on thermal induced acute pain, but synergistically analgesic for inflammatory induced acute and chronic pain.

KEYWORDS: Analgesia, nicotinic acetylcholine receptor, epibatidine, bupivacaine, spinal cord.

INTRODUCTION

Epibatidine is a highly potent non-selective agonist of neuronal nicotinic acetylcholine receptors (nAChRs).[1] Spinally administered epibatidine had analgesic effects on thermal acute pain[2], and formalin induced inflammatory pain.[3] To decrease toxicity of epibatidine, combination treatment with another agent should be considered. We have already shown that intrathecal epibatidine had antagonistic interaction with midazolam on thermal acute pain, but synergistic effects on acute inflammatory pain and additive effects on chronic inflammatory pain.[3] In the present study, we investigated interaction between intrathecally administered epibatidine and bupivacaine using the same rat models as our previous study.[3]

MATERIALS AND METHODS

After obtaining the approval of the Research Committee of the University of Tokyo, male Sprague-Dawley rats (280-300 g; Nippon Bio-Supply, Tokyo, Japan) were implanted with lumbar intrathecal catheters under halothane (2%) anesthesia. The experiment procedures are the same as our previous study.[3] Briefly, an 8.5 cm polyethylene catheter (PE-10; Clay Adams, Parsippany, NJ) was inserted caudally to the thoracolumbar level in the intrathecal space through atlanto-occipital membrane. The rostral part of the catheter was plugged with a 28-gauge steel wire and put through to the top of the skull. Only rats with normal motor function and behavior and increase in body weight seven days later were used for experiments. After the study, rats were euthanized under halothane 5% and the location of the catheter was confirmed anatomically and the data of the rats with mal location of the catheter was excluded, and another rat was added to fill the number of each group.

Drug preparation

The ED50s of epibatidine[3] and bupivacaine[4] were derived from our previous studies. The combination of 1/2, 1/4, 1/8, and 1/16 ED50s of epibatidine (Sigma, St. Louis, MO) and bupivacaine (Sigma, St. Louis, MO) were dissolved in 10 μL saline.

Tail-flick test

The tail-flick test was performed with the Tail-Flick Analgesia Meter (MK-330A; Muromachi Kikai Co. Ltd., Tokyo, Japan). Rats were placed in a clear plastic cage with their tails extending through a slot located of the rear of the cage. Thermal stimulation was given by a beam of high intensity light focused on the tail 2 to 3 cm

proximal to the end. The time between the start of the stimulation and tail withdrawal response was measured as a tail-flick latency. The cut-off time in the absence of a response was set to 14 seconds to prevent tissue injury of the tail. The test was done at 5, 10, 15, 30, 60, 90, 120, 180, and 240 minutes after drug injection. The data were shown as the % of maximum possible effect (% MPE): % MPE = (post-drug latency – pre-drug latency at time 0) X 100 / (cut-off time (14 sec) – pre-drug latency at time 0.

Formalin test

The formalin test was performed 10 minutes after intrathecal drug injection. Fifty μL of 5% formalin was injected subcutaneously into the dorsal surface of the right hind paw with a 30 G needle. Immediately after injection, the rat was placed in an open clear plastic chamber and their flinching or shaking paw response was observed for 60 minutes. The number of flinches was counted for 1 minute. Usually two phases were observed: phase 1, during 0 to 6 minutes after formalin injection; and phase 2, beginning about 10 minutes after injection with the interval of no flinches between both phases.

Side effects

Side effects were examined and judged as present or absent. Agitation was judged as spontaneous irritable movement, vocalization, or both. Allodynia-like behavior was judged as escape, vocalization, or both induced by lightly stroking the flank of the rat with a small probe. The placing or stepping reflex was evoked by drawing the dorsum of either hind paw across the edge of the table. Normal rats try to put the paw ahead into a position to walk. The righting reflex was assessed by placing the rat horizontally with its back on the table. Normally rats twist the body to an upright position immediately. Flaccidity was judged as muscle weakness by putting the forepaw 3 to 5 cm higher than the hind paw. Normal rats will walk up. Pinna or corneal reflex was examined with a paper string. When a string is put into the ear canal or touches the cornea, rats normally shake their heads. Behavioral side effects were checked simultaneously with the tail flick test.

Protocol

Each drug combination was administered intrathecally and after injection of the drug, the catheter was flushed with normal saline 10 μL to clear the dead space of the catheter. The ED50 was obtained using the maximum effects in the tail flick test and the area under the curve of the number of flinches in the formalin test. A total fractional dose value was calculated to describe the magnitude of the interaction as follows: (ED50 dose of drug 1 in combination) / (ED50 dose of drug 1 alone) + (ED50 dose of drug 2 in combination) / (ED50 dose of drug 2 alone). The value was normalized by assigning the ED50 value of each drug given alone as 1. Values near 1 suggest an additive interaction, values > 1 implies an antagonistic interaction, and values < 1 indicate a synergistic interaction. To compare the theoretical additive point with experimentally derived ED50, isobolographic analysis was used.

Data analysis

The data are shown as mean ± standard deviation or 95% confidential interval (CI). Statistical analysis was performed with the factorial analysis of variance (ANOVA) to compare the calculated ED50 values with theED50 of each agent alone and the theoretical additive values. A p value less than 0.05 was considered to be statistically significant.

RESULTS

We could not obtain ED50 of epibatidine + bupivacaine in the tail flick test (Table 1, Fig.1). In the formalin test, dose dependent decreases of number of flinches were observed (Fig. 2).

Synergistic effect was observed in both phase 1 (Fig.3, Table1) and phase 2 (Fig.4, Table 1) of the formalin test. Total fractional dose value could not be calculated in the tail flick test. Total fractional dose values were 0.47 (0.003 – 1.7) and 0.19 (0.03 – 1.9) in the phase 1 and 2 of the formalin test, respectively. No side effects were shown in the combination tested.

Table 1. ED50

	Tail flick	Formalin phase 1	Formalin phase 2
Bupivacaine (μg)	7.1 (3.5-13.8)	5.7 (3.5-8.8)	3.2 (1.7-6.3)
Epibatidine (ng)	32.0 (22.0-46.5)	38.0 (21.5-65.1)	27.1(10.4-43.5)
Bupivacaine in combination (μg)	N/A	1.2 (0.001-14)	0.3 (0.02-5.81)
Epibatidine in combination (ng)	N/A	9.8 (0.001-110)	2.6 (0.15-45.8)

ED50 values are shown as mean and 95% confidence interval (in parenthesis).
ED50s of epibatidine (1) and bupivacaine (2) are derived from our previous studies.

Figure legends

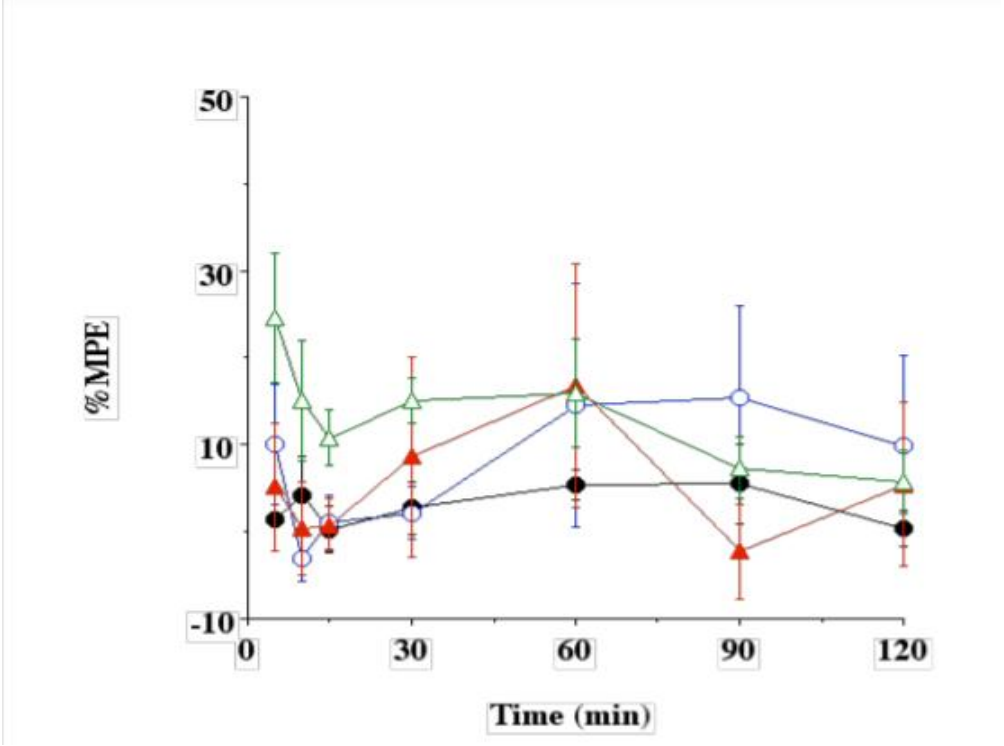

Figure 1: Tail flick test with bupivacaine + epibatidine.

%MPE, % of maximum possible effect; Bars indicate standard deviation. closed circle, saline; open circle, 1/16ED50; closed square, 1/8ED50; open square, 1/4ED50; closed triangle, 1/2ED50.

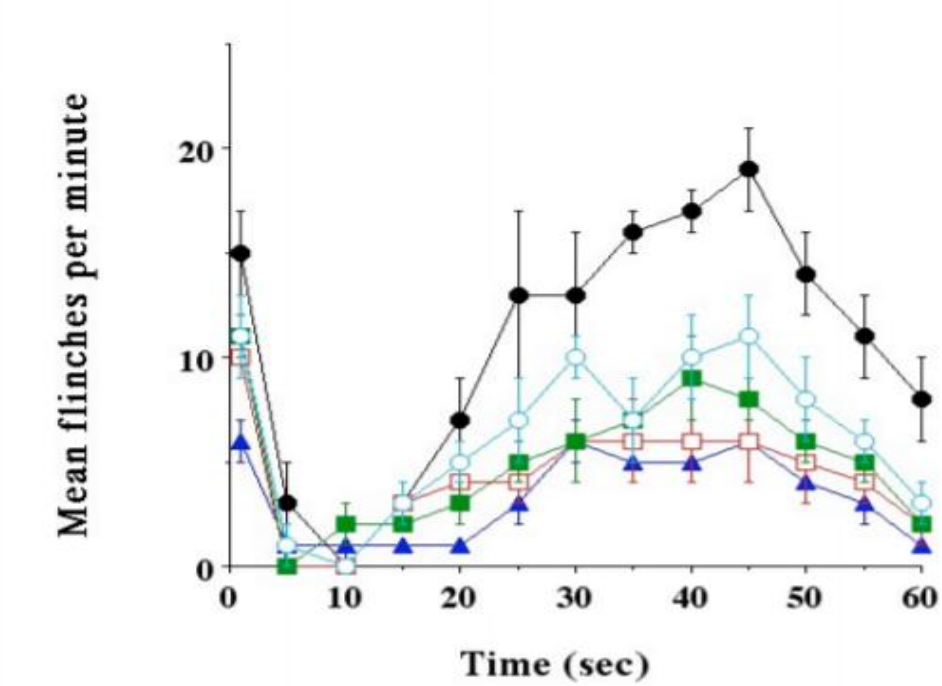

Figure 2: Formalin test with bupivacaine + epibatidine.

Bars indicate standard deviation. closed circle, saline; open circle, 1/16ED50; closed square, 1/8ED50; open square, 1/4ED50; closed triangle, 1/2ED50.

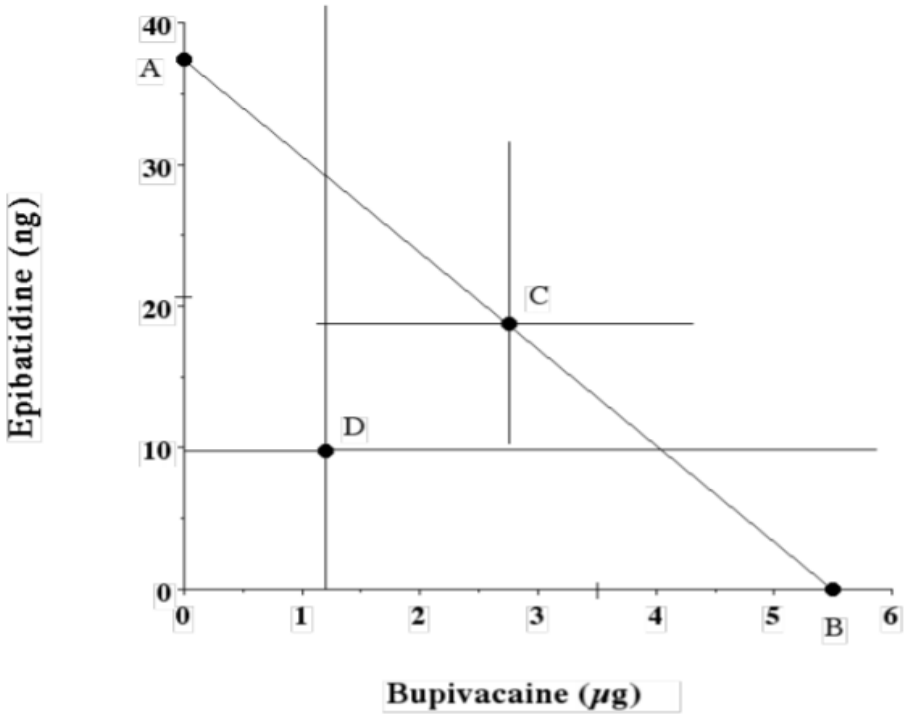

Figure 3: Isobolograph of the formalin test phase 1 with bupivacaine + epibatidine.

Bars indicate 95 % confidence interval. A, ED50 of epibatidine; B, ED50 of bupivacaine; C, theoretical additive point; D, calculated ED50 of bupivacaine + epibatidine.

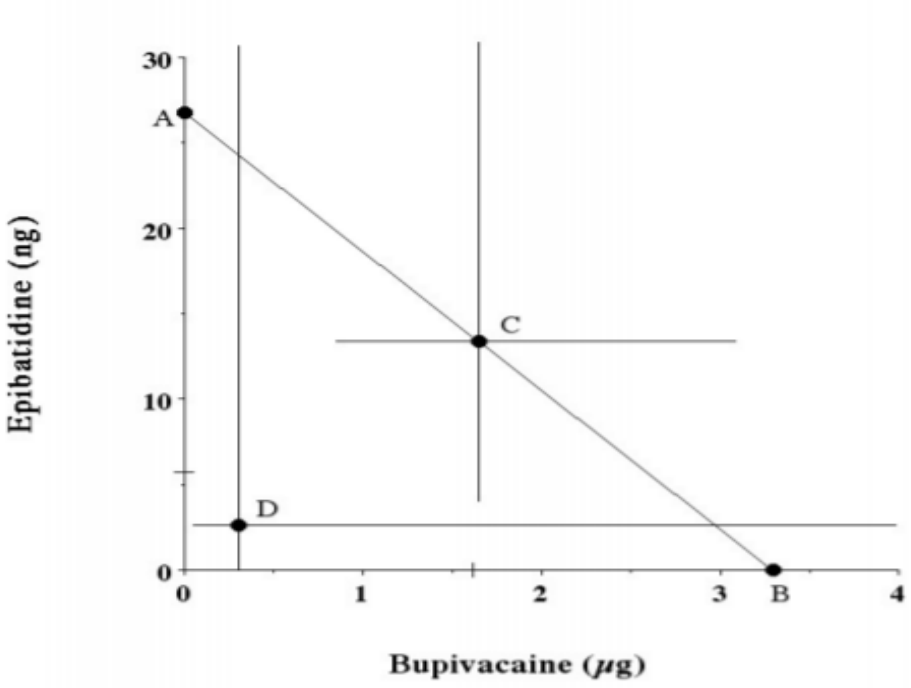

Figure 4: Isobolograph of the formalin test phase 2 with bupivacaine + epibatidine.

Bars indicate 95 % confidence interval. A, ED50 of epibatidine; B, ED50 of bupivacaine; C, theoretical additive point; D, calculated ED50 of bupivacaine + epibatidine.

DISCUSSION

Our results showed that intrathecally administered epibatidine and bupivacaine showed antagonism in the tail flick test, but synergistically analgesic in both phases of the formalin test.

The analgesic effect of intrathecal bupivacaine is mediated by block of neuronal sodium channels, potassium current, presynaptic muscarinic receptors, presynaptic calcium channels, and dopamine receptors.[4] Bupivacaine also inhibits N-methyl-D-aspartate (NMDA) induced currents in the spinal dorsal horn neurons.[5] Intrathecal bupivacaine significantly potentiated analgesic effect of intrathecal morphine, which might be due to the conformational change in the spinal opioid receptors by bupivacaine.[6]

Epibatidine showed both algesic and analgesic effects. The algesic effects of intrathecal epibatidine are mediated by α7 nAChRs, but analgesic effects are mediated by different subtypes of nAChRs.[7] Low doses of epibatidine reversed thermal and mechanical hyperalgesia in partial sciatic nerve-injured mice model by intrathecal administration.[8] This occurred through activation of neuronal nAChRs other than α4β2 subtype. Systemic and supraspinal epibatidine exerted an inhibitory effect on central pain transmitting pathways, while stimulatory effect is shown in the spinal cord.[9] According to Kahn et al.[10], intrathecal nicotinic agonists produce only a transient analgesia, while producing a more predominant algesic effect. They also showed that intrathecal epibatidine induced initial algesic response of short duration followed by transient analgesia.[11] From these reports, intrathecal epibatidine might act as either algesic or analgesic. However, in both tail flick test and formalin test, intrathecal epibatidine showed analgesic effects in the previous study.[3] Therefore, intrathecal epibatidine might act on nAChRs other than α7 and α4β2 subtypes in thermal or inflammatory induced pain.

Epibatidine activates distinct subtypes of nicotinic receptors in spinal cord. These receptors might be expected to be presynaptic and control the release of excitatory amino acids.[12] Intrathecal epibatidine induces dose-dependent increases in the spinal release of aspartate and glutamate[11], which might induce algesic effects. Genzen et al.[13] showed a mechanism of nicotinic analgesia, at least in part, depends on both short and long-term modulation of γ-amino butyric acid (GABA)ergic synaptic transmission in the spinal cord dorsal horn.

Intrathecal epibatidine had antagonistic interaction with midazolam on thermal acute pain, but synergistic effects on acute inflammatory pain and additive effects on chronic inflammatory pain in our previous study.[3] The present study also showed antagonistic interaction between intrathecal epibatidine and bupivacaine on thermal acute pain, but synergistic effects on acute and chronic inflammatory pain. However, there is no data to show the interaction between nAChRs and bupivacaine. When combined with bupivacaine, epibatidine induced glutamate release much more than acting on nAChRs or modulating GABAergic transmission in thermal acute pain, while epibatidine might act on nAChRs or increase GABA in chemical induced pain. These might be clarified in the future studies.

In conclusion, intrathecal bupivacaine and epibatidine had antagonistic effects on thermal induced acute pain, but synergistically analgesic for inflammatory induced acute and chronic pain.

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