Epinephrine Potentiates the Analgesic and Antidepressant Effects of Amitriptyline as a Result of Stimulation of the Gastric Mucosal Afferents

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Intramuscular amitriptyline in the minimum effective dose causes maximum analgesic and antidepressant effect and significant sedation in rats. Combined injection of amitriptyline with epinephrine in the threshold doses (ineffective if used alone), $^1/_{10}$ and $^1/_{30}$ minimum effective doses, respectively, leads to the development of the maximum analgesic and antidepressant effect, but causes no sedative side effect. This potentiation is mediated by stimulation of afferents in the gastric mucosa with epinephrine.

Key Words: *epinephrine; amitriptyline; pain; depression; sedation*

High-dose (10-20 mg/kg) amitriptyline significantly reduces behavioral depression in Porsolt's test and suppresses pain sensitivity in the tail-flick test in rats [6,7,10]. However, the side effect of amitriptyline in these doses in rats is sedation manifesting in significant reduction of horizontal motor activity in the open field (OF) test [6,9,10].

Intramuscular injection of high minimally effective doses (MED) of epinephrine leads to the maximum analgesic and antidepressant effects as a result of stimulation of the subdiaphragmatic gastric vagus afferents [2,4]. However, high doses of epinephrine also cause severe bradycardia associated with simultaneous activation of cardiac vagus efferents [3,8].

Combined injection of epinephrine with polyvinylpyrrolidone and of epinephrine with cholecystokinin in threshold doses, ineffective if used alone and constituting $^1/_{10}$-$^1/_{25}$ MED, leads to development of the maximum analgesic and antidepressant effects, but causes no bradycardia. It was found that this potentiation is mediated by stimulation of afferents in the gastric mucosa (GM) with epinephrine [5]. We hypothesized that stimulation of GM afferents with threshold epinephrine doses leads to potentiation of the antidepressant and analgesic effects of amitriptyline not accompanied by sedative side effects.

We studied the potentiating effects of threshold epinephrine doses on the analgesic, antidepressant, and sedative effects of amitriptyline.

MATERIALS AND METHODS

Experiments were carried out at 10.00-16.00 on outbred male albino rats (180-200 g; 8-10 per group). Epinephrine and amitriptyline (Sigma) and combination of amitriptyline with epinephrine in the threshold dose (0.012 mg/kg; $^1/_{10}$ MED) were injected intramuscularly 30 min before testing. Controls were injected with 0.2 ml distilled water intramuscularly.

The analgesic effects of the drugs were evaluated from prolongation of the tail-flick latency [2]. Antidepressant effect was studied in modified 2-day Porsolt's test [4], the duration of immobilization was evaluated during 10-min forced swimming.

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For quantitative and analgesic activities, MED of amitriptyline, epinephrine, and amitriptyline+epinephrine causing the maximum antidepressant (immobilization <40 sec) and analgesic effects (tail-flick latency>30 sec) were calculated [2,4].

The role of GM afferents in the mechanism of epinephrine potentiation of the analgesic and antidepressant effects of amitriptyline was studied in anesthesized animals: 30 min before combined injection of these drugs the rats received intragastrically 1% local anesthetic (lidocaine; 0.5 ml) or ganglionic blocker (hexamethonium; 0.2 mg/kg) [2,4].

The sedative effects of the drugs were studied in the OF test [1,6]. The animals were placed in the center of a square illunibated field (1×1 m) and the number of crossed squares (horizontal activity) and rearing postures (vertical activity) were recorded for 3 min.

Sedative activity of amitriptyline, epinephrine, and their combination in doses causing maximum analgesic and antidepressant effects was evaluated by the decrease in horizontal and vertical motor activities in comparison with the control.

The data were processed statistically using Student's test.

RESULTS

Epinephrine in MED (0.12-0.13 mg/kg) caused maximum analgesic and antidepressant effect in rats and virtually did not change horizontal and vertical motor activities in the OF test (Table 1), this indicating that epinephrine had no sedative effect.

Injection of amitriptyline in MED to rats also led to the development of maximum analgesic and antidepressant effects (Table 2). It is known that injection of amitriptyline in high doses causes not only analgesic and antidepressant effects in rats, but also a sedative side effect significantly reducing horizontal motor activity in the OF test [6,9,10]. Amitriptyline in a dose of 10 mg/kg significantly reduced horizontal (4.8 times; $p<0.05$) and vertical (2.3 times) activities of rats in the OF test (Table 1). Hence, injection of amitriptyline in MED to rats caused not only maximum analgesic and antidepressant effects, but also a significant sedative effect in the OF test.

TABLE 1. Effects of Intramuscular Drugs on the Rat Behavior in OF Test

Group		Number of crossed squares	Number of rearing episodes
Distilled water, ml/kg		24.3±2.7	8.1±1.1
Amitriptyline	10 mg/kg	5.1±0.6*	3.5±0.4
	0.2 mg/kg	25.0±3.0	8.3±1.1
Epinephrine	0.12 mg/kg	19.3±2.3	7.7±1.0
	0.012 mg/kg	27.0±3.2	9.0±1.1
Amitriptyline (0.2 mg/kg)+ epinephrine (0.012 mg/kg)		24.0±2.9	8.5±1.2

Note. *$p<0.05$ compared to the control.

Injections of epinephrine and amitriptyline in threshold doses ($^1/_{10}$-$^1/_{30}$ MED) led to the development of slight analgesic and antidepressant effects (2-5% of the maximum) without sedative effect in the OF test.

Combined injection of amitriptyline with epinephrine in the threshold dose of 0.012 mg/kg ($^1/_{10}$ MED) allows significant ($p<0.05$) reduction of amitriptyline MED causing maximum analgesic and antidepressant effects (by 31 and 29 times, respectively; Table 2), to a level of the threshold doses causing just minor analgesic and antidepressant effects, if used alone. The combination of threshold amitriptyline and epinephrine doses caused no sedative effect, virtually not reducing the motor activity of rats in the OF test in comparison with the control (Table 1).

Preliminary lidocaine anesthesia of GM and hexamethonium blockade of intramural GM ganglia suppressing epinephrine stimulation of the GM afferents [2,4] completely abolished the maximum anal-

TABLE 2. Amitriptyline MED, Causing Analgesic and Antidepressant Effects in Rats

Group	MED, mg/kg	
	analgesic effect in the tail-flick test	antidepressant effect in Porsolt's test
Amitriptyline	9.1±1.2	11.2±0.9
Amitriptyline+epinephrine	0.300±0.034*	0.410±0.046*
Amitriptyline+epinephrine+lidocain	12.6±1.4	13.3±1.6
Amitriptyline+epinephrine+hexamethonium	12.0±1.3	12.6±1.4

Note. *$p<0.05$ compared to amitriptyline in MED.

gesic and antidepressant effects of amitriptyline combinations with epinephrine (Table 2). Hence, epinephrine stimulation of GM afferents underlies the mechanism of potentiation of analgesic and antidepressant effects of amitriptyline. The antidepressant and analgesic effects of intramuscular high-dose epinephrine are due to epinephrine stimulation of the subdiaphragmatic gastric vagus afferents [2,4]. Presumably, weak central antidepressant and analgesic effects caused by threshold doses of amitriptyline are potentiated by stimulation of the subdiaphragmatic gastric vagus afferents by the threshold doses of epinephrine.

The results suggest that stimulation of GM afferents with epinephrine in threshold doses leads to potentiation of the antidepressant and analgesic effects of amitriptyline in the threshold doses to a level of the maximum effects caused by amitriptyline MED, but without the development of sedative side effect.

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