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Original Research Article

Beta-adrenergic receptor blockers effects on the antinociceptive action of imipramine against thermal induced pain in albino mice

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Abstract

Introduction: Tricyclic antidepressant have been shown to be effective in treatment of pain of varying etiology, monoaminergic system seems to be implicated in this phenomena. This research examines the role of beta-adrenergic receptor blockers on the antinociceptive effect of imipramine in albino mice using thermal model of pain.

Methods: Different groups of five animals each were injected intraperitoneal by different doses of imipramine only (2.5, 7.5,15, 30 mg/kg), atenolol (2 mg/kg), propranolol (6mg/kg), or the combination of the different doses of imipramine with the fixed dose of atenolol or propranolol. The degree of analgesia was measured as an increase in reaction time to pain in the hot plate one hour after drugs injections.

Results: Imipramine produced dose dependent increase in reaction time from 129% with the lowest dose to 196% with the highest dose. One-way ANOVA analysis has shown that the addition of a fixed dose of propranolol antagonized significantly the increase in reaction time to 75% with the lowest dose and 118.9% with the highest dose of imipramine. On the other hand, atenolol failed to antagonize significantly the increase in reaction time induced by imipramine.

Conclusion: Imipramine has a significant analgesic effect on albino mice in the hot plate test. The antinociceptive action of imipramine seems to be of central origin and possibly mediated, at least in part, by beta adrenergic receptors, as this analgesic effect can be blocked by propranolol, a centrally acting non-selective beta adrenergic receptor antagonist, but not with atenolol which blocks only the peripheral beta receptors.

Key-words:

Imipramine, atenolol, propranolol, analgesia, thermal nociception.

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1. Introduction

It is generally agreed that adrenergic receptors have a major role in the modulation of pain, where descending adrenergic tracts especially from locus coeruleus (LC) were found to be inhibitory to central pain pathways [1]. Many studies have demonstrated that activation of spinal α_2-adrenergic receptors exerts a strong antinociceptive effect [1], where spinal clonidine blocked thermal and capsaicin-induced pain in healthy human volunteers [2]. Activation of α_2-adrenergic receptors has been shown to inhibit nociceptive transmission at the level of the spinal cord through presynaptic activity, inhibiting release of excitatory neurotransmitters from primary afferent terminals, as well as through postsynaptic sites [3]. Moreover, activation of α_1-adrenergic receptors caused depolarization of GABA interneurons [4], demonstrating an additional mechanism of enhancing inhibition. Activation of spinal α_1-adrenergic receptors also enhances responses of dorsal horn neurons to noxious inputs [5].

On the other hand, the role of beta receptor in nociception is still not clear, where a study in 1970 [6], and later in 1983 [7], showed that beta adrenergic receptor agonists had a marked antinociceptive effect by an action within the mouse peritoneal. Furthermore, convincing evidence that stimulation of beta$_2$-adrenergic receptors by nortriptylline have a major role in the pain management effect in neuropathic pain [8].

As it is not clear if central or peripheral beta-adrenergic receptors are involved in the antinociceptive effect of tricyclic antidepressants (TCAs), we decided to investigate the effect of centrally acting beta-receptor antagonist (propranolol) and peripherally acting beta-receptor antagonist (atenolol) on the antinociceptive effect of imipramine against thermal pain.

2. Materials & Methods

Animals used

Albino mice weighing 20-35 grams were obtained from the animal house of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, University of Tripoli one week before the experiments. The animals were kept at a normal room temperature of 20 to 25 °C with open access to food and water. All experiments were done between 9 am to 2 pm.

Drugs used

Imipramine HCL tablet (Actavis, Barnstaple, UK), containing 10 mg of imipramine was dissolved in saline to make a final concentration of 3 mg/ml. After filtration, this stock solution was diluted to prepare solutions of different concentrations.

Propranolol ampule (Mibe GmbH Arzneimittel, Brehna, Germany) containing

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1 mg/ml propranolol was diluted with normal saline. Atenolol tablet (50 mg/tablet) (AstraZeneca-Egypt) was dissolved in saline to make a final concentration of 0.2 mg/ml.

Treatment groups

All drugs were given in saline by the intraperitoneal route (IP) in a volume of 1ml/100 grams body weight. Animals were divided into 5 groups.

- The first group was divided into subgroups of 5 animals each, each subgroup was given either 2.5 mg/kg, 7.5 mg/kg, 15 mg/kg or 30 mg/kg imipramine.
- The second group of five animals was given 6 mg/kg propranolol.
- The third group of five animals was given 2 mg/kg atenolol.
- The fourth group was divided and treated as the first group, but propranolol (6 mg/kg) was given with the specific dose of imipramine each on different side of the peritoneal cavity.
- The fifth group was divided and treated as the first group, but atenolol (2 mg/kg) was given with the specific dose of imipramine each on different side of the peritoneal cavity.

The degree of analgesia was measured one hour after drugs injections using hotplate.

Method of inducing nociception

Hot-plate Analgesia Meter (model – DS 37 manufactured by Soael Milan-Italy) was used as a mean of inducing thermal pain as previously described [9]. The plate temperature was held at a set point by electronic thermostat set at (55±0.2) °C [10 & 11]. Mice were brought to the testing room and allowed to acclimatize for 10 minutes before the test begins. Mouse was placed on the hot plate and the latency to respond with a hind paw lick or jump (whichever comes first) was measured in seconds. The mouse was immediately removed from the hot plate and returned to its home cage. If a mouse did not respond within 30 seconds, the test was terminated and the mouse was removed from the hot plate. Baseline measurement for each mouse was taken just prior to drug administration (pre-treatment values or self-control) and again 60 minutes after drug administration.

The experiments were performed according to a protocol approved by the Animal Care Committee of the Department of Pharmacology and Clinical Pharmacy, University of Tripoli.

Data presentation and statistical analysis

The degree of antinociception for each mouse (except for the group that received propranolol or atenolol only) was expressed as the percentage increase in reaction time (RT) in seconds calculated according to the formula (% increase in RT = [T*100/C]), where C is the RT before

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treatment and T is the RT after treatment. For the group that received atenolol or propranolol only the comparison was made between the means of the RT before and after treatments.

Data generated from the above studies were statistically analyzed with Microsoft excel software. Results for each group were expressed as Mean ± S.E. One-way analysis of variance (ANOVA) was used to see the differences in the effects among the different treatments (imipramine with or without beta-blocker), and the paired or unpaired t-tests to determine which population means were different. A *P* value of <0.05 was considered statistically significant.

3. Results

3.1. Antinociceptive effect of imipramine

Different doses of imipramine (2.5 mg to 30 mg/kg) produced a significant dose dependent increase in the reaction time in the hot plate test as measured by ANOVA ($P<0.05$). The highest dose of imipramine produced a significantly stronger analgesic effect when compared with the lowest dose (unpaired t-test $P<0.05$) (Figure 1).

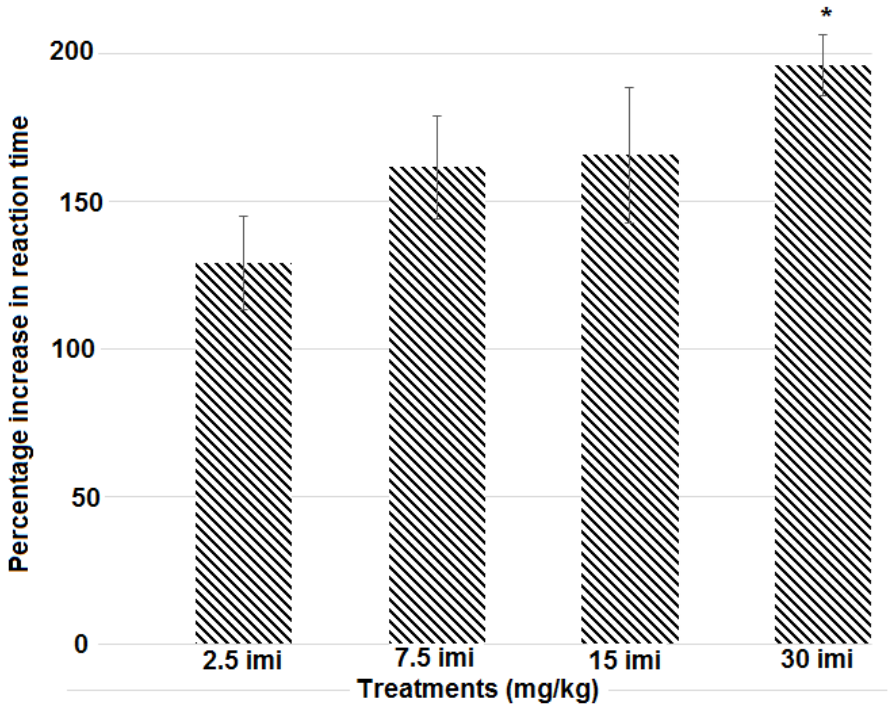

Figure 1: The percentage increase in reaction time produced by imipramine (imi) in hot plate test. *$P<0.05$ using unpaired student t-test between the two groups that were given either 2.5 mg/kg or 30 mg/kg imipramine. Values are means ± SE of five animals.

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3.2. Antinociceptive effect of propranolol and atenolol

Comparing pre-treatment and post-treatment reaction times, propranolol (6.0 mg/kg) and atenolol (2.0 mg/kg) produced a non-significant increase in the reaction time in the hot plate test measured by the paired t-test. (Figure 2).

3.3. Antinociceptive effect of the combined treatment with propranolol and imipramine.

Fixed dose of propranolol (6.0 mg/kg) had antagonized the antinociceptive effect of the different doses of imipramine measured by ANOVA (P<0.05). Although this antagonism was apparent for all doses of imipramine, it was significant only with the lowest (2.5 mg/kg) and the highest (30 mg/kg) doses of imipramine as measured by unpaired t-test (P<0.05) (Figure 3).

3.4. Antinociceptive effect of the combined treatment with atenolol and imipramine.

Fixed dose of atenolol (2.5 mg/kg) had no significant effect on the antinociceptive effect of the different doses of imipramine as measured by ANOVA (P>0.05). However, atenolol had antagonized significantly the antinociceptive effect of the highest dose (30 mg/kg) of imipramine as measured by unpaired t-test (P<0.05) (Figure 4).

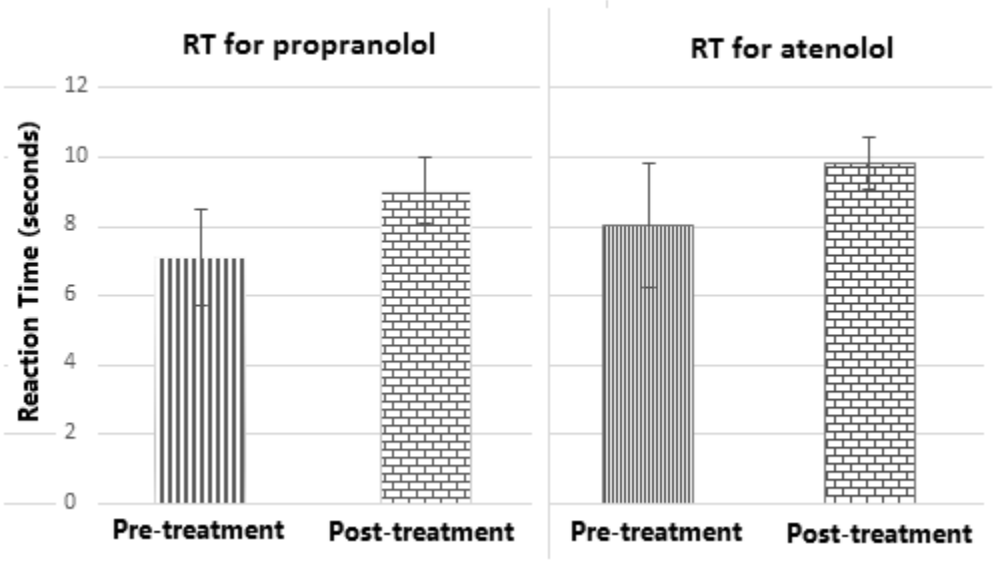

Figure 2: The pre-treatment and post-treatment reaction time produced by propranolol and atenolol in hot plate test. Values are means ± SE of five animals.

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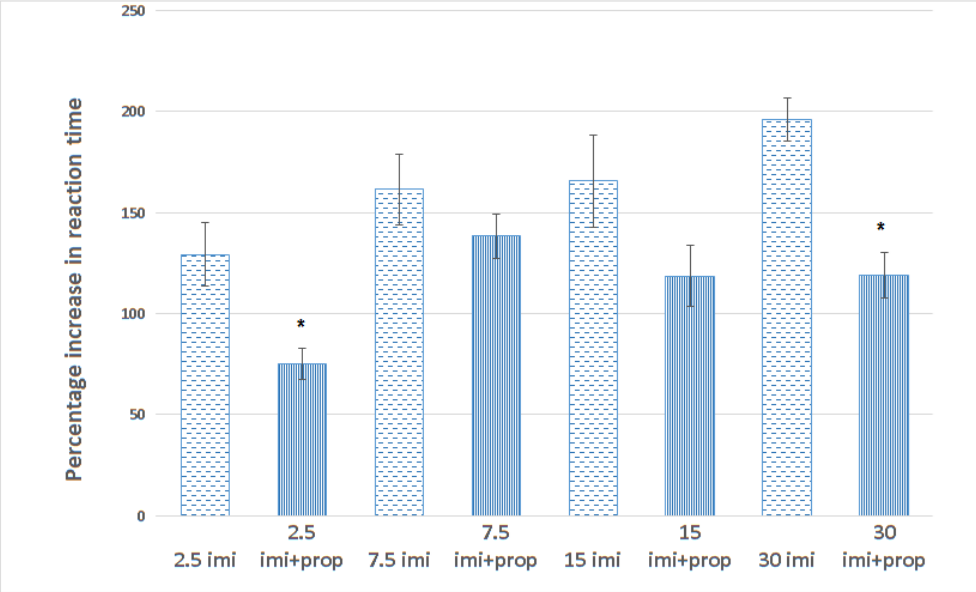

Figure 3: The percentage increase in reaction time produced by imipramine only (imi) or the combined treatment with a fixed dose of propranolol (prop.) and imipramine. Values are means ± SE of five animals. (*P<0.05 using unpaired student t-test between the group that received imipramine only or imipramine plus propranolol).

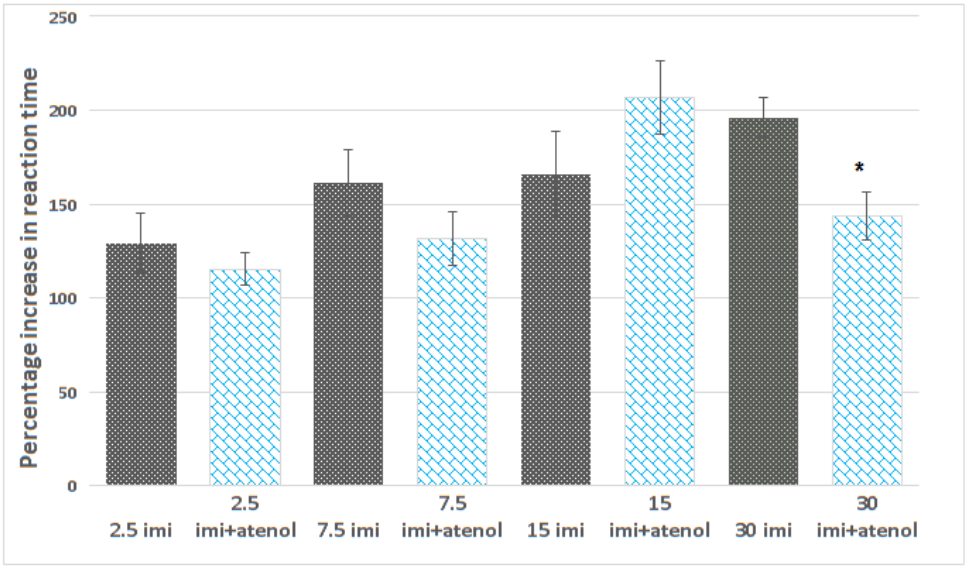

Figure 4: The percentage increase in reaction time produced by imipramine only (imi) or the combined treatment with a fixed dose of atenolol (aten.) and imipramine. Values are means ± SE of five animals. (*P<0.05 using unpaired student t-test between the group that received imipramine only or imipramine plus propranolol).

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4. Discussion

Imipramine produced dose dependent analgesic effect against hotplate. One-way ANOVA analysis have shown that the addition of a fixed dose of propranolol antagonized the analgesic effect of different doses of imipramine. On the other hand, atenolol failed to antagonize the analgesic effect of imipramine using one-way ANOVA. When the un-paired t-test was used, it was found that the antagonism by propranolol took place with the lowest and highest dose of imipramine, with a non-significant trend of antagonism with the middle doses. These results are in good agreement with previous studies, where penbutolol (a non-selective beta-blocker with central effects) antagonized the analgesic effect of another TCA desipramine [12]. Moreover, both CGP 20712A, a selective beta$_1$-adrenoceptor antagonist, and ICI 118551, a selective beta$_2$-adrenoceptor antagonist inhibited the analgesic effect of desipramine and nortriptyline in the hotplate test [12].

This study shows that the analgesic effect of imipramine in hot plate test was inhibited by propranolol (centrally acting non-selective beta blocker), but not with atenolol (selective beta$_1$-antagonist with no central effect). The main reason for this disparity is probably due to the difference in the lipophilic nature of propranolol and it is ability to cross the blood brain barrier [13], in contrast to atenolol with it is hydrophilic nature [14]. Thus, propranolol antagonized the analgesic effect of imipramine by blocking beta-adrenergic receptors centrally. In accordance with this proposal, it was shown that stimulation of central beta adrenergic receptors increased the latency in the licking response of mice in the hot plate [15], implicating a role for these receptors in modulating pain processes. Moreover, beta-adrenergic receptors are closely related to serotonergic [16] and opioid [15] neural systems that are directly implicated in pain modulation, which in turn, may have participated indirectly in the mechanism of action of imipramine.

5. Conclusions

This study has shown that the antinociceptive action of imipramine in hot plate seems to be of central origin and possibly mediated, at least in part, by beta-adrenergic receptors, as this analgesic effect can be blocked by propranolol, a centrally acting non-selective beta adrenergic receptor antagonist, but not with atenolol which have mainly peripheral effects.

6. Limitation of the Study

This study examined the effect of beta adrenergic receptor blockers on the antinociceptive action of imipramine only. Other antidepressants should have been included for comparison. This is a suggestion for further research in this field.

Key Message: Central and not peripheral Beta-adrenergic receptors are involved directly or indirectly in the antinociceptive effect of imipramine in the thermal model of pain.

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7. References

[1] Ossipov MH, Dussor GO, Porreca F. Central modulation of pain. The Journal of clinical investigation. 2010;120(11):3779-87.

[2] Eisenach JC, Hood DD, Curry R. Intrathecal, but not intravenous, clonidine reduces experimental thermal or capsaicin-induced pain and hyperalgesia in normal volunteers. Anesthesia & Analgesia. 1998;87(3):591-6.

[3] Pertovaara A. Noradrenergic pain modulation. Progress in neurobiology. 2006;80(2):53-83.

[4] Gassner M, Ruscheweyh R, Sandkühler J. Direct excitation of spinal GABAergic interneurons by noradrenaline. PAIN®. 2009;145(1):204-10.

[5] Budai D, Harasawa I, Fields HL. Midbrain periaqueductal gray (PAG) inhibits nociceptive inputs to sacral dorsal horn nociceptive neurons through α2-adrenergic receptors. Journal of neurophysiology. 1998;80(5):2244-54.

[6] Gardella JL, Izquierdo JA. The analgesic action of catecholamines and of pyrogallol. European journal of pharmacology. 1970;10(1):87-90.

[7] Bentley GA, Newton SH, Starr J. Studies on the antinociceptive action of α-agonist drugs and their interactions with opioid mechanisms. British journal of pharmacology. 1983;79(1):125-34.

[8] Bentley GA, Starr J. The antinociceptive action of some β-adrenoceptor agonists in mice. British journal of pharmacology. 1986;88(3):515-21.

[9] Elhwuegi AS, Hassan KM. The analgesic effect of different antidepressants combined with aspirin on thermally induced pain in Albino mice. Libyan Journal of Medicine. 2012;7(1).

[10] O'Callaghan JP, Holtzman SG. Quantification of the analgesic activity of narcotic antagonists by a modified hot-plate procedure. Journal of Pharmacology and Experimental Therapeutics. 1975;192(3):497-505.

[11] Carter RB. Differentiating analgesic and non-analgesic drug activities on rat hot plate: effect of behavioral endpoint. Pain. 1991;47(2):211-20.

[12] Micó JA, Gibert-Rahola J, Casas J, Rojas O, Serrano MI, Serrano JS. Implication of β 1-and β 2-adrenergic receptors in the antinociceptive effect of tricyclic antidepressants. European neuropsychopharmacology. 1997;7(2):139-45.

[13] Rodriguez-Romaguera J, Sotres-Bayon F, Mueller D, Quirk GJ. Systemic propranolol acts centrally to reduce conditioned fear in rats without

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impairing extinction. Biological psychiatry. 2009;65(10):887-92.

[14] Agon P, Goethals P, Haver D, Kaufman J. Permeability of the blood-brain barrier for atenolol studied by positron emission tomography. Journal of pharmacy and pharmacology. 1991;43(8):597-600.

[15] Brochet D, Micó JA, Martin P, Simon P. Antinociceptive activity of beta-adrenoceptor agonists in the hot plate test in mice. Psychopharmacology. 1986;88(4):527-8.

[16] Cowen PJ. A role for 5-HT in the action of antidepressant drugs. Pharmacology & therapeutics. 1990;46(1):43-51.

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ملخص باللغة العربية

دور حاصرات مستقبلات بيتا الأدرنرجية على عمل الإيميبرامين المضاد للألم الناجم عن الألم الحراري في الفئران البيضاء

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الملخص

مقدمة: ثبت أن مضادات الاكتئاب ثلاثية الحلقات فعالة في علاج الألم المتفاوت الأسباب وذلك عبر تأثيرهم على الأمينات الأحادية في الجهاز العصبي المركزي. هذا البحث يتناول دور حاصرات مستقبلات بيتا الأدرنرجية على تأثير الإيميبرامين المضاد للأحساس بالألم في الفئران البيضاء باستخدام نموذج الألم الحراري.

الطرق: مجموعات مختلفة كل منها تتكون من خمسة حيوانات حقنت صفاقيا بجرعات مختلفة من إيميبرامين فقط (2.5، أو 7.5 أو 15 أو 30 ملغ/كلغ)، أتينولول فقط (2 ملغ/كلغ)، بروبرانولول فقط (6 ملغ/ كلغ)، أو مزيج من الجرعات المختلفة من الإيميبرامين مع جرعة ثابتة من أتينولول أو بروبرانولول. وقد تم قياس درجة التسكين ساعة واحدة بعد حقن الأدوية باستخدام نموذج الألم الحراري (السطح الساخن).

النتائج: نتج عن الإميبرامين تأثير معتمد على الجرعة مضاد للألم باستعمال السطح الساخن. وقد أظهرت تحليل الأنوفا في اتجاه واحد أن إضافة جرعة ثابتة من بروبرانولول منع التأثير المسكن للجرعات المختلفة من الإيميبرامين. من ناحية أخرى، فشل الأتينولول من منع التأثير المسكن للإيميبرامين.

الخلاصة: إميبرامين له تأثير فعال مسكن للألم في الفئران البيضاء باستعمال السطح الساخن. ويبدو أن التأثير المسكن للألم لإيميبرامين مركزي المنشأ، وربما جزئيا، من خلال تاثيره على مستقبلات بيتا الأدرينرجية حيث أن هذا التأثير ثم منعه من قبل بروبرانولول الذي يعتبر حاصر لمستقبلات بيتا الإدرنرجية وله تأثير مركزي، ولم يتم منعه من الأتينولول الذي يقوم بإحصار مستقبلات البيتا الأدرنرجية الطرفية فقط.

الكلمات المفتاحية:

إميبرامين، أتينولول، بروبرانولول، تسكين، حس الألم الحراري.

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