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Combined use of tertiary amine parasympathomimetics with a quaternary amine parasympatholitic – a new perspective to use parasympathomimetic drugs for systemic analgesia

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The interactions on antinociception between a muscarinic agonist arecoline (arec), an anticholinesterase physostigmine (physo) which both cross CNS, and a peripherally acting antimuscarinic hyoscine-*N*-butyl bromide (hyo), were assessed by tail flick test in mice. All drugs were administered intraperitoneally (i.p.). While hyoscine-*N*-butyl bromide (0.15 and 4.00 mg/kg, i.p.) did not produce antinociception, physostigmine salicylate (0.3 mg/kg, i.p.) and arecoline hydrobromide (8.00 mg/kg, i.p.) exerted significant antinociceptive effect. In combined applications, physo + hyo (0.075 + 0.15; 0.15 + 0.30; 0.30 + 0.60 mg/kg) and arec + hyo (1.00 + 0.50; 2.00 + 1.00; 4.00 + 2.00; 8.00 + 4.00 mg/kg), respectively, produced significant antinociception and the tail flick latencies produced by physo 0.30 + hyo 0.60 mg/kg and arec 8.00 + hyo 4.00 mg/kg were not significantly different from those of physo 0.30 mg/kg and arec 8.00 mg/kg, respectively, showing that hyo did not antagonise the antinociceptive effects of physo and arec. We believe that combining an centrally acting cholinergic drug applied systemically with a peripherally acting (quaternary amine) antimuscarinic compound might be used as an effective analgesic in clinical practice.

1. Introduction

Because of the unwanted effects of classical opioids such as respiratory depression and drug dependence, the search for new potent analgesics has been continuing. One of the approaches directed to this is to create mixed acting opioids with antinociceptive efficacy lower than that of opioid agonists and the other way is to use opioids combined with analgesic adjuvants such as amphetamine [1] and ephedrine [2, 3], a procedure which is not common in current clinical practice.

It was demonstrated in animal studies that parasympathomimetic drugs have antinociceptive effects comparable with opioids [4]. However, because of their side effects, these drugs could not be administered systemically. The intrathecal injection of neostigmine has been tried clinically to produce analgesia, but even in this application the drug produces prominent side effects such as severe emesis and fecal incontinence [5, 6]. In addition, intrathecal application necessitates skilled persons and has many drawbacks [7]. Therefore, the intrathecal administration of neostigmine is not suitable for routine clinical pain therapy.

The antinociceptive effect of parasympathomimetic drugs result from a stimulation of central muscarinic receptors [8]. It was thought that while stimulating the central muscarinic receptors through systemically administered parasympathomimetic drugs which easily cross the blood-brain barrier the peripheral effects can be antagonised by antimuscarinics having a quaternary amine group therefore not being able to enter the central nervous system. For this purpose, a muscarinic agonist (arecoline, arec), an anticholinesterase agent (physostigmine, physo), and an antimuscarinic drug (hyoscine-*N*-butyl bromide, hyo) were investigated in mice by the tail flick test.

2. Investigations and results

The effects of our treatment on the tail flick latency differences in mice are presented in the Fig.

In single drug applications, physo (0.30 mg/kg) and arec (8.00 mg/kg) produced significant tail flick latency compared with SF ($P < 0.05$). However, hyo (0.15 and 4.00 mg/kg) did not cause any significant effect.

Combinations, 0.075; 0.15; 0.30 mg/kg physo + 0.15; 0.30; 0.60 mg/kg hyo, and 1.00; 2.00; 4.00; 8.00 mg/kg arec + 0.5; 1.0; 2.0; 4.0 mg/kg hyo, produced significant tail flick latency compared with saline ($P < 0.05$). In addition, the differences between 0.30 mg/kg physo + 0.60 mg/kg hyo and 0.30 mg/kg physo alone were not significant ($P > 0.05$). Combination of 8.00 mg/kg arec with 4.00 mg/kg hyo did not produce a significant change in the tail flick latencies compared with 8.00 mg/kg arec alone ($P > 0.05$).

One of the animals died after injection of 0.30 mg/kg physo.

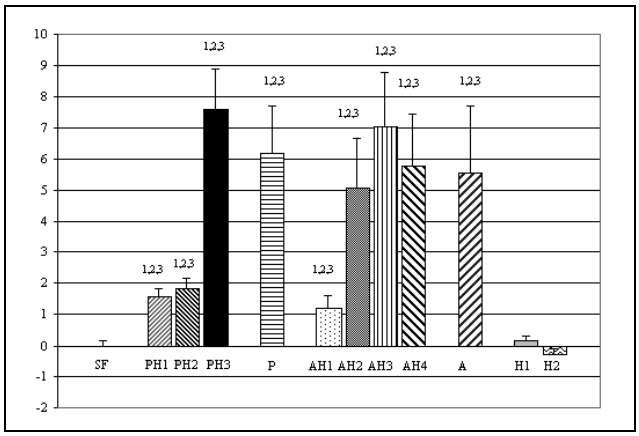

Fig.: The tail flick latency of physostigmine (P), arecoline (A) and hyoscine-*N*-butyl bromide (H) in mice on tail flick test. Ordinate:latency difference in seconds before and after treatment. In each group n = 10, except 0.30 mg/kg physo group (n = 9). Vertical lines show s.e. mean. SF: Saline; PH1: 0.075 mg/kg physo + 0.15 mg/kg hyo; PH2: 0.15 mg/kg physo + 0.30 mg/kg hyo; PH3: 0.30 mg/kg physo + 0.60 mg/kg hyo; P: 0.30 mg/kg physo; AH1: 1.00 mg/kg arec + 0.50 mg/kg hyo; AH2: 2.00 mg/kg arec + 1.00 mg/kg hyo; AH3: 4.00 mg/kg arec + 2.00 mg/kg hyo; AH4: 8.00 mg/kg arec + 4.00 mg/kg hyo; A: 8.00 mg/kg arec; H1: 0.15 mg/kg hyo; H2: 4.00 mg/kg hyo. $P < 0.05$ as compared to saline (1), 0.15 mg/kg hyo (2) and 4.00 mg/kg hyo (3).

3. Discussion

Our findings indicate that the co-administration of hyo with physo or arec does not significantly alter the antinociceptive effects of these drugs. This shows that, while centrally acting parasympathomimetics exert analgesic effects, their peripheral effects may be blocked by peripherally-acting antimuscarinics.

Lauretti and Lima [9] have investigated the interaction between intrathecal neostigmine and intravenous hyo on postoperative patients. They reported that an addition of hyo facilitates the relief of abdominal pain. The tail flick method used in the present study represents the somatic pain [10], accordingly in order to determine the effects on visceral pain, it is necessary to use different methods.

Our findings show that hyo alone does not significantly effect the tail flick latency in mice indicating that the drug is not effective against somatic pain. It was reported that atropine produced analgesia at lower doses (s.c., 1–100 μg/kg) and hyperalgesia at higher doses (s.c., 5 mg/kg) in mice in the hot plate test and the drug induced this effect by acting centrally [11]. Therefore, we conclude that hyo, a peripherally acting drug, is devoid of any antinociceptive effect.

As far as we know, our study is unique in that it investigates the interaction between physo and arec with hyo on antinociception. Sheardown reported that methscopolamine (s.c., 0.1 and 1.0 mg/kg) did not antagonise the analgesic effect of oxotremorine (s.c., 0.1 mg/kg) [4]. This finding in this paper is consistent with our study.

As a consequence, it seems to be a useful approach to combine systemically-acting parasympathomimetic drugs with peripherally-acting antimuscarinic compounds in order to provide powerful analgesia. However, to reach a definite conclusion, advanced studies must be carried out to investigate the side effects caused by the influence of this combination.

4. Experimental

4.1. Animals

Swiss albino mice of both sexes (n = 120), weighing 27.91 ± 3.86 were used. Each group consisted of 5 male and 5 female animals. We made 12 groups. The animals were housed in a climate-controlled room maintained at about 21 °C with approximately 50% relative humidity. Lighting was on a 12-h light/dark cycle (lights on 7.00 a.m.), with standard laboratory chow (Aytekinler, Turkey) and water available ad libitum. The study was approved by the University Ethics Committee and all tests were performed in accordance with the recommendations and policies of the international associations for the handling and use of experimental animals [12].

4.2. Tail flick test

Tail flick latencies weres obtained using an analgesiameter (May 9604-A, Turkey). Radiant heat was focused on a spot 1 cm from the tip of the tail, and the latency until the mouse flicked its tail was recorded (cut-off time 15 s). Beam intensity was adjusted to give a tail flick latency between 2–3 s in control animals. The animals were restrained during trials by means of Plexiglas cylinder 3 cm in diameter and 10 cm length. The latency was measured two times in each animals. The first measurements were made prior to injections and the second were performed after the injections had been completed. The differences between the two measurements (tail flick data) were subjected to the statistical evaluation.

4.3. Drugs and administration route

Hyoscine-N-butyl bromide (Buscopan® inj., 20 mg/ml, Boehringer, Ingelheim, Turkey) was diluted in saline (0.9% NaCl) to final concentrations of 0.015; 0.030; 0.060; 0.1; 0.2; 0.4 mg/ml. Physostigmine salicylate (Serva Feinbiochemical, Heidelberg, Germany) and arecoline hydrobromide (Sigma Chemical Co., St. Louis, USA) were dissolved in saline. All doses refer to the base. Drug solutions were freshly prepared and injected intaperitoneally in a volume of 10 ml/kg, 20 min before hyo and physo and 10 min before arec. In control group saline was used. In combined groups different doses of physo + hyo (3 groups), and arec + hyo (4 groups) were applied. We used physo, arec and two doses of hyo in single groups. In combined applications, drug solutions were applied in the separate regions. The animals in control and single drug groups were also subjected two injections, saline + saline or drug solution + saline respectively.

4.4. Statistical analysis

Tail flick data (in seconds as means ± s.e) were subjected to Student's t test (two-tailed) for unpaired data which was used to detect statistical differences between the groups. For the test, a P value <0.05 was considered statistically significant.

References

1 Forrest, W. H. Jr.; Brown, B. W. Jr.; Brown, C. R.; Defalque, R.; Gold, M.; Gordon, H. E.; James, K. E.; Katz, J.; Mahler, D. L.; Schroff, P.; Teutsch, G.: N. Eng. J. Med. **296**, 712 (1977)
2 Tekol, Y.; Tercan, E.; Esmaoglu, A.: Acta. Anaesthesiol. Scand. **38**, 396 (1994)
3 Tekol, Y.; Koramaz, S.: Analgesia **2**, 113 (1996)
4 Sheardown, M. J.; Shannon, H. E.; Swedberg, M. D.; Suzdak, P. D.; Bymaster, F. P.; Olesen, P. H.; Mitch, C. H.; Ward, J. S.; Sauerberg. P.: J. Pharmacol. Exp. Ther. **281**, 868 (1997)
5 Eisenach, J. C.; Hood, D. D.; Curry, R.: Anesth. Analg. **85**, 842 (1997)
6 Klamt, J. G.; Garcia, L. V.; Prado, W. A.: Anaesthesia **54**, 27 (1999)
7 Collins, V. J.: Principles of Anesthesiology, 2. Ed. p. 659, Lea and Febiger. Philadelphia, 1976
8 Bartolini, A.; Ghelardini, C.; Fantetti, L.; Malcangio, M.; Malmberg-Aiello, P.; Giotti, A.: Br. J. Pharmacol. **105**, 77 (1992)
9 Lauretti, G. R.; Lima, I. C. : Anesth. Analg. **82**, 617 (1996)
10 Miyamoto, H.; Saito, Y.; Kirihara, Y.; Hara, K.; Sakura, S.; Kosaka, Y.: Anesth. Analg. **90**, 136 (2000)
11 Ghelardini, C.; Malmberg-Aiello, P.; Giotti, A.; Malcangio, M.; Bartolini, A.: Br. J. Pharmacol. **101**, 49 (1990)
12 Canadian Council on Animal Care:Guide to the Care and Use of Experimental Animals (Volume I), Ottawa 1984

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