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Antinociceptive Effect of *R*-(+)-Hyoscyamine on the Conjunctival Reflex Test in Rabbits

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ABSTRACT—*R*-(+)-Hyoscyamine (1–10 μg/kg, s.c.) dose-dependently increased the local anesthetic effect of procaine (50 μg/ml) and lidocaine (50 μg/ml) in the conjunctival reflex test in the rabbit. This potentiating effect is completely prevented by the M_1 antagonist dicyclomine (10 mg/kg, s.c.). The intensity of *R*-(+)-hyoscyamine antinociception was comparable to that induced by morphine (2 mg/kg, s.c.) and minaprine (15 mg/kg, s.c.), used as analgesic reference drugs. In the same experimental conditions, the *S*-(−)-enantiomer of atropine (0.1–10 μg/kg, s.c.), was completely ineffective. The present results confirm the ability of *R*-(+)-hyoscyamine to produce a paradoxical antinociceptive effect mediated by a cholinergic mechanism not only in rodents but also in the rabbit.

Keywords: Atropine, *R*-(+)-Hyoscyamine, Antinociception, Local anesthetics, Cholinergic system

Very low doses of the antimuscarinic compound atropine were able to induce central cholinergic antinociception in the mouse hot-plate and abdominal constrictions tests and in the rat paw pressure and tail flick tests (1). The atropine-induced increase in the pain threshold was attributable to the *R*-(+)-enantiomer of atropine, *R*-(+)-hyoscyamine, since *S*-(−)-hyoscyamine was ineffective in all antinociceptive tests used (2). More recently, Ghelardini et al. (3), investigating the antinociceptive effect of atropine, demonstrated, by using microdialysis techniques, that *R*-(+)-hyoscyamine at analgesic doses increased the acetylcholine release from the rat cerebral cortex in vivo. The cholinergic hypothesis for the mechanism of analgesic action of *R*-(+)-hyoscyamine is also confirmed by the prevention of its antinociceptive effect exerted by M_1-selective muscarinic antagonists (3).

On the bases of the above-mentioned results, we thought it worthwhile to further investigate the antinociceptive properties of *R*-(+)-hyoscyamine. For this purpose, we studied the ability of systemically injected *R*-(+)-hyoscyamine to potentiate the local anesthetic effect induced by procaine or lidocaine on the rabbit conjunctival reflex test.

MATERIALS AND METHODS

Animals

Male albino rabbit (2.5–3.0 kg) from Morini (San Polo d'Enza, Italy) were used. All experiments were carried out according to the guidelines of the European Community Council on animal care.

Rabbit conjunctival reflex test

The test was conducted according to the method described by Donatelli and Buffoni (4). The external side of the rabbit eye was stimulated with a cat whisker to induce the conjunctival reflex and consequently the closure of the palpebrals. One drop of a 50 μg/ml solution of procaine or lidocaine was placed into rabbit eyes as local anesthesia. The number of stimuli necessary to provoke the conjunctival reflex was counted in the absence or in the presence of increasing doses of *R*-(+)-hyoscyamine s.c. injected 15 min before local anesthetic administration.

Drugs

The following drugs were used: *R*-(+)-hyoscyamine, prepared in the Department of Pharmaceutical Sciences of the University of Florence (Italy) according to the method described by Gualtieri et al. (5); *S*-(−)-hyoscyamine hydrobromide, lidocaine hydrochloride, procaine hydrochloride (Sigma, Milan, Italy); minaprine hydrochloride (Cantor-Midy, Montpellier, France); dicyclomine hydrochloride (LePetit, Milan, Italy); morphine hydrochloride (USL 10/D; Florence, Italy). Drugs, with the exception of *R*-(+)-hyoscyamine, were dissolved immediately before use in isotonic (NaCl 0.9%) saline solution. *R*-(+)-hyoscyamine was dissolved in an amount of 0.1 M HCl which exceeded the stoichiometric requirement by 10% and then diluted with the saline. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml/kg subcutaneously (s.c.), with the exception of lidocaine and procaine which were prepared as 50 μg/ml solutions.

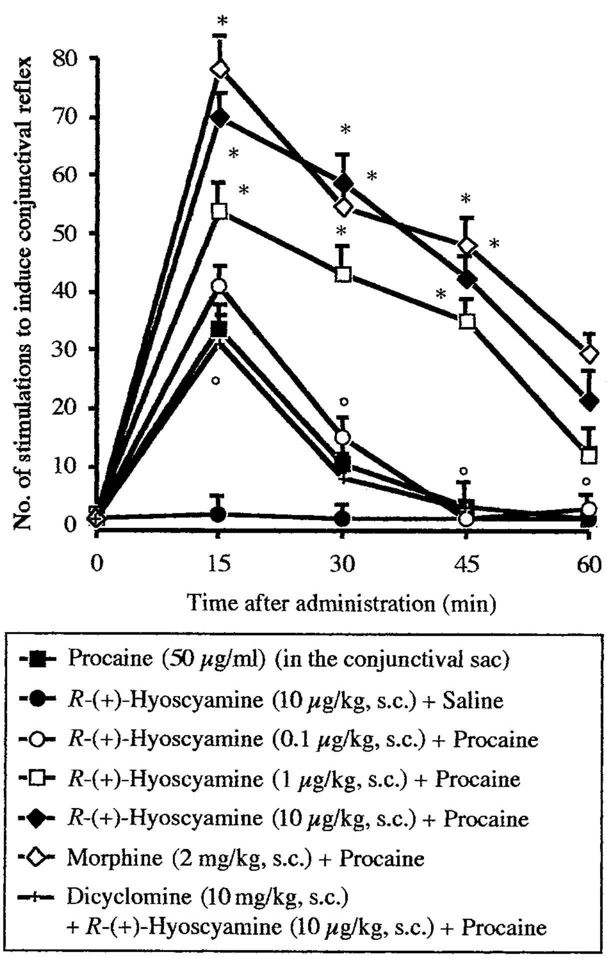

Fig. 1. Effects of *R*-(+)-hyoscyamine in comparison with morphine on the local anesthesia induced by procaine in the rabbit conjunctival reflex test and antagonism by dicyclomine (10 mg/kg, s.c.) of the *R*-(+)-hyoscyamine induced potentiation in the same test. Each point represents the mean of at least 4 rabbits. Dicyclomine, morphine, *R*-(+)-hyoscyamine and procaine were injected respectively 45, 30, 15 and 5 min before the test. *$P<0.05$, in comparison with rabbits treated with procaine alone. °$P<0.05$, in comparison with *R*-(+)-hyoscyamine (10 μg/kg, s.c.)+procaine-treated group.

Statistical analyses

Results are given as the mean±S.E.M.; analysis of variance, followed by Scheffe's F procedure for post-hoc comparison, was used to verify the significance between two means. P values of less than 0.05 were considered significant. Data were analyzed with the StatView for Macintosh (1992) computer program (Abacus Concepts, Inc., Berkeley, CA, USA).

RESULTS

R-(+)-Hyoscyamine (1−10 μg/kg, s.c.), dose-dependently increased the local anesthetic effect of procaine (Fig. 1) and lidocaine (Fig. 2) instilled in the conjunctival sac. The *R*-(+)-hyoscyamine potentiating effect was observed starting 15 min after administration and persisted for up to 45 min and then slowly diminished. This effect is completely prevented by the M_1 antagonist dicyclomine injected at the dose of 10 μg/kg, s.c. 30 min before *R*-(+)-hyoscyamine (Fig. 1). The intensity of *R*-(+)-

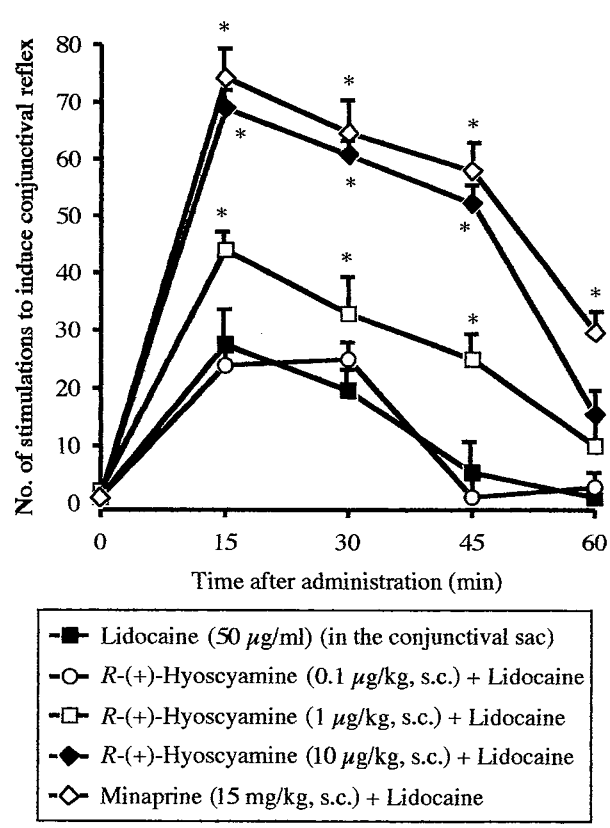

Fig. 2. Effects of *R*-(+)-hyoscyamine in comparison with minaprine on the local anesthesia induced by lidocaine in the rabbit conjunctival reflex test. Each point represents the mean of at least 4 rabbits. Minaprine, *R*-(+)-hyoscyamine and lidocaine were injected respectively 30, 15 and 5 min before the test. *$P<0.05$, in comparison with rabbits with lidocaine alone.

hyoscyamine antinociception was comparable to that induced by morphine (2 mg/kg, s.c.; Fig. 1) and minaprine (15 mg/kg, s.c.; Fig. 2). Morphine and minaprine when injected in the absence of local anesthetics did not exhibit any effect (data not shown). *R*-(+)-Hyoscyamine, at the dose of 0.1 μg/kg, s.c., was completely ineffective in potentiating the local anesthethic effect (Fig. 1). At the highest active dose, *R*-(+)-hyoscyamine did not modify the number of stimuli necessary to provoke the conjunctival reflex in the absence of local anesthetics (Fig. 1).

In the same experimental conditions, the *S*-(−)-enantiomer of atropine (0.1−10 μg/kg, s.c.), was completely ineffective in potentiating the local anesthetic effect (data not shown).

The *R*-(+)-hyoscyamine-induced increase of the local anesthetic effect was obtained in rabbits without any visible change in the normal behavior of animals (data not shown).

DISCUSSION

The present study illustrates the paradoxical analgesic effect of the active enantiomer of atropine, *R*-(+)-hyoscyamine. In agreement with our results, other paradoxical effects of atropine were previously observed by Ferguson-Anderson (6) and Brown (7) who reported that in humans, low doses of atropine increased gastric contraction frequency and amplitude and decreased heart rate. Bülbring (8) and Ghelardini et al. (1, 3) described, respectively, the ability of atropine and *R*-(+)-hyoscyamine to potentiate cholinergic transmission in vitro at the isolated neuromuscular junction of rat phrenic hemidiaphragm and in the longitudinal muscle strip of guinea pig through the block of the presynaptic muscarinic autoreceptor.

R-(+)-Hyoscyamine is able to potentiate the local anesthetic effect of lidocaine and procaine in rabbits. We can rule out that *R*-(+)-hyoscyamine is endowed with local anesthetic properties since it is devoid of any effect when given alone. Moreover, a local anesthetic action of *R*-(+)-hyoscyamine can be excluded since it does not reduce (in the range between $10^{-14}-10^{-3}$ M) the electrically evoked contractions of isolated guinea pig and rat phrenic nerve-hemidiaphragm (9).

The potentiating effect of *R*-(+)-hyoscyamine can, therefore, be ascribed only to an analgesic activity. Hotovy (10) reported that the first analgesic dose of atropine, injected parenterally in the rabbit, is 30 μg/kg, whereas the lowest dose able to potentiate local anesthetics is 1 μg/kg. Therefore, the potentiating activity of *R*-(+)-hyoscyamine is expressed out at doses lower than those active as an analgesic. This is not a peculiar effect of *R*-(+)-hyoscyamine, but it is a common property to numerous well-known analgesic drugs such as morphine, tricyclic antidepressants and salmon calcitonin (11). This property can be useful for the clinical employment of the analgesic drugs since the compounds endowed with potentiating properties can be used, in association with other analgesics, at lower doses, reducing the incidence of side effects. The potentiating effect of local anesthetics produced by *R*-(+)-hyoscyamine was obtained without any modification of the rabbits' normal behavior.

Since the potentiating effect exerted by *R*-(+)-hyoscyamine on the responses to the local anesthetics is completely abolished by the pretreatment with dicyclomine, the involvement of a cholinergic mechanism of action can be postulated. This hypothesis is also supported by the observation that oxotremorine, a direct colinomimetic, at the dose of 100 μg/kg, s.c., is able to increase the local anesthetic effect of procaine instilled in the conjunctival sac (data not shown). The present results confirm the ability of *R*-(+)-hyoscyamine to produce a paradoxical antinociceptive effect through an amplification of cholinergic neurotransmission, not only in rodents but also in the rabbit.

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