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ScienceDirect
Phytomedicine 14 (2007) 65–69
Phytomedicine
www.elsevier.de/phymed

Effect of Thymol on the spontaneous contractile activity of the smooth muscles

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Received 19 April 2006; accepted 10 July 2006

Abstract

Effects of Thymol on the spontaneous contractile activity (SCA) have been found in in vitro experiments with circular smooth-muscle strips (SMAs) from guinea pig stomach and vena portae. Thymol was found to possess an agonistic effect on the α_1-, α_2- and β-adrenergic receptors. Its spasmolytic effect is registered at doses higher than 10^{-6} M.

Thymol in a dose of 10^{-4} M inhibits 100% the SCA of the SMAs and reduces the excitatory effect of 10^{-5} M ACH to 35%.

It is assumed that Thymol has an analgesic effect through its action on the α_2-adrenergic receptors of the nerve cells. By influencing the β-adrenergic receptors in the adipose cells, it is possible to induce increased synthesis of fatty acids and glycerol, which is a prerequisite for increased heat release.

Keywords: Smooth muscle fibres; Thymol; Thymus vulgaris; α_1-, α_2- and β-adrenergic receptors; Papaverine; Pain; Clonidine

Introduction

There are descriptions in the literature of antibacterial (Didry et al., 1994), antiphlogistic (Anamura, 1989; Wagner et al., 1986), spasmolytic (Cabo et al., 1986) and other effects of Thymol. There are no studies, however, on whether Thymol possesses a specific effect on certain receptors. Such research is difficult because Thymol has spasmolytic effects, similar to Papaverine, but it is known that the specific effects are usually manifested in very small concentrations of the agents. The ability to study the effects, therefore, requires a concentration range of the agents between the effectiveness of the specific and the non-specific effects of usually 1–2 orders (10–100 times). The aim of this study was to determine whether Thymol possesses other effects in addition to those described previously in the literature. The answer to this question can better clarify the multilateral effects, especially when applied externally. The model chosen was the effect of Thymol on the spontaneous contractile activity (SCA) of smooth-muscle strips (SMS) from the stomach and vena portae of guinea pigs.

Materials and methods

Agonists and antagonists were used in the in vitro studies (Sigma, St. Louis, MO, USA). Acetylcholinchloride (ACH) was obtained from Dispersa (Germering,

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0944-7113/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2006.11.010

Germany) Papaverinehydrochloride and Thymol came from Merck (Darmstadt, Germany). The purity of Thymol was greater than 99%, as measured by gas chromatography.

The solubility of Thymol in water 1 g/l – approximately 6.6 mM (Budavari et al., 1989)-is sufficient for the experiments carried out. In our experiments, we used Thymol concentrations from 10^{-8} to 10^{-4} M. The volume of the bath in which the SMAs were placed was 15 ml. Only 0.227 ml of the 1 g Thymol dissolved in 1 l of water was necessary to reach the concentration of Thymol in the bath with 10^{-4} M Ringer solution. The measurements of the SCA of the SMAs and the influence of Thymol were made according to the method of Golenhofen et al. (1976).

In order to take into account deviations in the measured values of the SCA, which are specific to the preparations, we represent the dose curves of the effect of the measured excitation always as percentage of the maximum contractile activity of SMAs under the effect of 10^{-5} M acetylcholine (ACh). Data were analyzed using the StatSoft statistics program (4.5; StatSoft Inc., Microsoft, USA). Comparison between two groups was performed using the Student's *t*-test. To compare three or more groups, analysis of variance (ANOVA) was employed. Standard deviation for this method is below 5%. Seven measurements were performed for each experiment.

Experimental results

Fig. 1 shows the dose–effect curve of Thymol (10^{-8}–10^{-4} M). The effect of Thymol from 2×10^{-8} to 10^{-6} M induces a clear excitation of the SCA of the SMSs from guinea pig stomach, which reached about 15% of the maximum contractile activation (10^{-5} M ACh). Further increase of the Thymol concentration leads to inhibition of these excitatory effects. At 10^{-4} M Thymol, even the SCA of SMS is inhibited. The tone of the SMAs is close to the zero line. These inhibitory processes are manifested more slowly than the excitatory effects registered with the lower Thymol doses.

Fig. 2 (upper) shows the curves of the effect of Thymol on SCA of SMS under normal conditions and with preliminary blocking of the α_1- and α_2-adrenergic receptors with 10^{-5} M Benextramine. When the α_1- and α_2-adrenergic receptors are blocked, the excitatory effects of Thymol on SCA of SMS are completely blocked. Fig. 2 (lower) presents the curves of the effect of Thymol on SCA of SMS under normal conditions and with preliminary blocking of the β-adrenergic receptors with 10^{-5} Alprenolole. The excitatory effect of Thymol on the SCA of SMS increases. It is necessary to identify the significance of these excitatory effects of Thymol on the SMAs of α_1- and α_2-adrenergic receptors. Our experiment demonstrates that the α_2-adrenergic receptors are much better represented in the circular SMAs from the corpus part of guinea pig stomach than the α_1-adrenergic receptors. The opposite effect is observed in the longitudinal smooth muscles of the vena portae.

Fig. 3 (upper) presents the dose curves of the effect of Thymol (10^{-8}–10^{-6} M) on the SCA of the vena portae under normal conditions and with preliminary blocking of the α_1-adrenergic receptors. The excitatory action of Thymol is entirely eliminated only by blocking of the α_1-adrenergic receptors. Fig. 3 (lower) shows the dose curves of the effect of Thymol on the SCA of vena portae under normal conditions and with preliminary blocking of the α_2-adrenergic receptors with 10^{-5} M Rauwolscine. It can be seen that the excitatory effects of Thymol on the SCA of vena portae are insignificantly influenced.

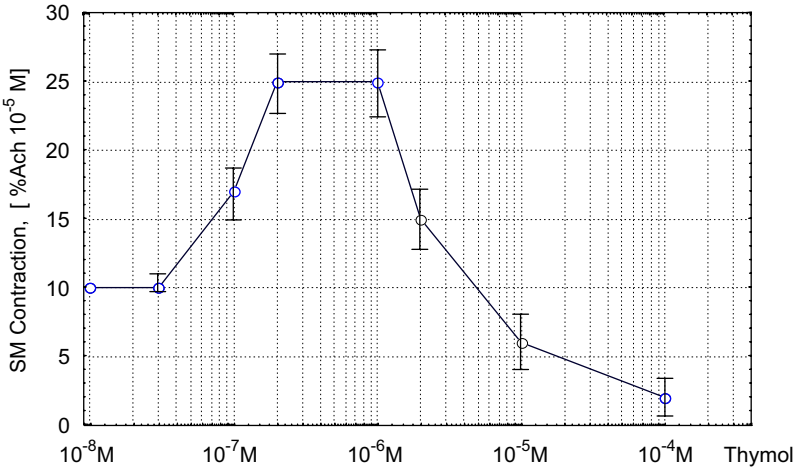

Fig. 1. Dose–effect curve of Thymol (10^{-8}–10^{-4} M) on the spontaneous contractile activity of smooth-muscle strips from guinea pig stomach.