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THE EFFECT OF PHARMACOLOGICALLY ACTIVE COMPOUNDS FROM *GINKGO BILOBAE* EXTRACT ON CONTRACTILITY OF RABBIT DUODENUM AND ILEUM

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Findings that there are pharmacologically active compounds in the Ginkgo bilobae extract recently provoked numerous investigations of their action on the receptors of the autonomous nervous system. The purpose of the present study was to determine the influence of gingko extract on the contractility of the longitudinal smooth muscle layer in the wall of the rabbit duodenum and ileum using the method of Magnus. Results clearly show that gingko extract causes a dose dependent tonus decrease of duodenal and ileal smooth muscles in the rabbit. It also reduces the stimulation of spontaneous activity of smooth muscles caused by acetylcholine, the reduction being greater in ileum compared to duodenum.

Key words: Ginko bilobae extract, duodenum, ileum, smooth muscle tonus, acetylcholine

INTRODUCTION

Ginkgo bilobae, plant from *Ginkgoaceae* family, attracts human attention over 2000 years. In traditional Chinese medicine ginkgo extracts were used for the treatment of lung disorders. Today they are used for memory improvement, therapy of depression, and prevention of disorders caused by oxidative stress (Sasaki *et al*., 2002). During the last 10 years biochemical analyses revealed components extracted from fresh ginkgo leaves which contain 3 groups of specific compounds exerting pharmacological effects. The largest portion among them (22-27%) are ginkgo-flavonolglycosides: quecetin, campherol and isochamnetine (Hansel *et al.*, 1993, DeFeudis *et al.*, 1991). This group of polyphenolic compounds is characterized by the presence of C_{15} skeleton in which two benzolic rings are connected over a C_3 bridge (C_6-C_3-C_6). Other constituents of the ginkgo extract are terpenes containing lactonic ring (ginkgolides A, B, C, J and M, and bilobaides) and specific acids (ginkgoinate and ginkgolinate).

Active substances from the ginkgo extract influence primarily muscarinic, and on a smaller scale, adrenergic receptors. It has been proven that the use of ginkgo extract impedes the reduction of the number of muscarinic and α_2

adrenergic receptors, at the same time improving the uptake of choline in synapses. It also quenches the toxic free radicals (Sasaki *et al*., 2002; Shulz *et al*., 2001).

The purpose of this study is to determine the effects of the ginkgo extract on the contractility of the longitudinal smooth muscle layer in the wall of rabbit duodenum and ileum.

MATERIAL AND METHODS

Extract preparation

In this study we used a German preparation *GINGIUM®*. Its solution was standardized so that 1 ml contained 40 mg of dry extract from Ginkgo bilobae leaves (35 – 67:1). Extraction was performed using 60 vol.% acetone. One milliliter of the preparation contained 8.8 – 10.8 mg ginkgo-flavonol glycosides and 2.0 – 2.8 mg of terpens with lactonic ring (ginkgolides and bilobaides).

Experimental animals and design

Ten mature Chinchilla rabbits, males and females, weighting around 3 kg each, aged 2.5 to 3 months were used. After the sacrifice 20-30 mm long stripes of the duodenal and ileal walls were excised from each animal and prepared using the method of Magnus (Radenković, 1995). Strips were placed in the bath for isolated organs filled with Tyrod's solution. One end of the strip was fixed to the holder on the bottom of the bath and the other end to the isotonic transducer of the physiograph which registered the degree of contraction. Tonus of the isolated strips was measured as length compared to the basal (zero) line. Tonal decrease was denoted as negative, and increase as positive numeric factor, where the value of 1 meant 100% change in length.

The experimental model consisted of two groups of treatments: in the first group the effect of adding ginkgo extract in non-cumulative, increasing logarithmic concentrations (0.006 g/l, 0.02 g/l and 0.06 g/l) was observed.

In the second experimental group we first examined the effect of increasing logarithmic concentrations of acetylcholine (ACh) on the contraction of longitudinal smooth muscle layer of the duodenal and ileal wall. ACh was applied as follows:

Group A = 6.6×10^{-9} mol/l   Group B = 2.2×10^{-8} mol/l
Group C = 6.6×10^{-8} mol/l   Group D = 2.2×10^{-7} mol/l

After intermission of at least half hour, the effects of ACh were examined on the same strips, this time in the presence of 0.2 g/l ginkgo extract.

Every treatment was performed on at least 8 strips from the same part of the small intestine, originating from different animals.

Statistical analysis

Significance of difference among treatments was tested using ANOVA and student's t-test in SPSS for Windows v.8.

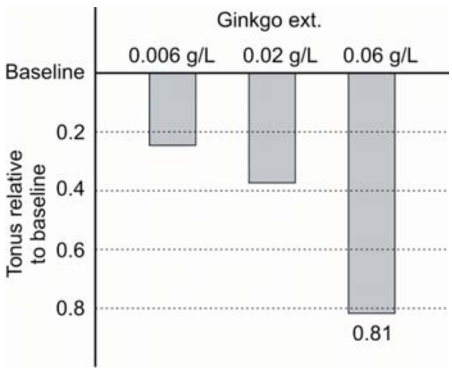
RESULTS

Ginkgo extract produced a significant ($p<0.01$) dose dependent tonus reduction of the duodenal wall sections (Graph 1). Maximal reduction rate, by the factor -0.81 compared to the basal line, was recorded in the group of sections treated with highest ginkgo extract concentration of 0.06 g/l. The duration of the inhibition of spontaneous duodenal smooth muscle activity was 2-3 minutes in sections treated with 0.006 g/l, while in those treated with 0.06 g/l inhibition lasted 10 minutes.

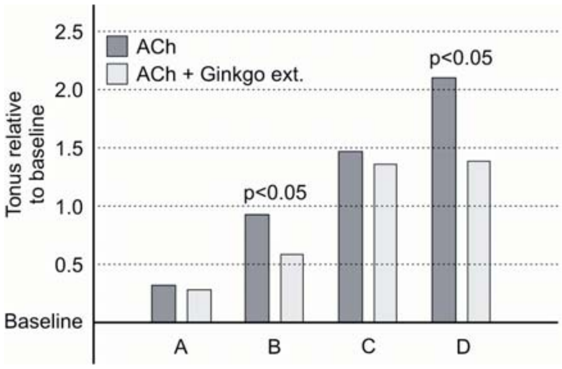
In the absence of the ginkgo extract, logarithmic increasing doses of acetylcholine (ACh), caused a significant ($p<0.05$) tonus increase of the duodenal wall sections. Maximal increase, by the factor of +2.2 compared to baseline, was recorded in the group of sections treated with the highest ACh concentration of $2.2x10^{-7}$ mol/l. When ginkgo extract (0.2 g/l) was added to the solution, significant reduction ($p<0.05$) of above mentioned ACh effect was recorded in groups of sections B and D. The effects of ACh and ginkgo extract on the tonus of duodenal wall sections are shown on Graph 2.

In the sections of the ileal wall smooth muscles, ginkgo extract caused a significant ($p<0.01$) dose dependent tonus reduction (Graph 3). Maximal reduction, by the factor -0.84 compared to baseline, was recorded in sections treated with ginkgo extract concentration of 0.06 g/l.

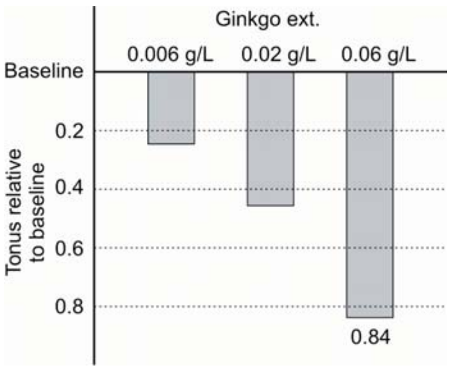
ACh in logarithmic increasing doses, in the absence of the ginkgo extract, produced a significant ($p<0.05$) tonus increase of the ileal smooth muscles. Maximal increase rate (by the factor +2.3 compared to basal value) was recorded in sections treated with highest ACh concentration of $2.2x10^{-7}$ mol/l. However, in the presence of ginkgo extract (0.2 g/l), effects of ACh on the ileal wall tonus were significantly lower ($p<0.01$) in all sections. The effects of ACh and ginkgo extract on the tonus of ileal wall sections are shown on Graph 4.



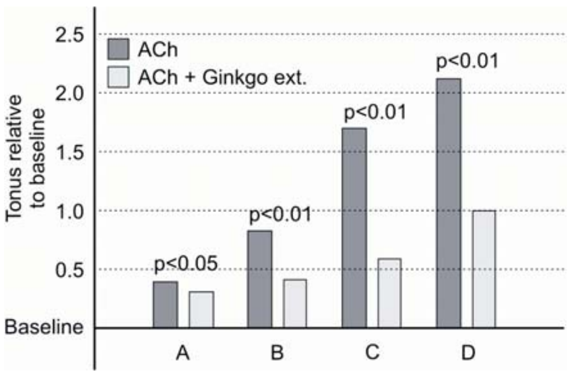
Graph 1. Dose dependent tonus reduction of the duodenal wall in the presence of ginkgo extract in different concentrations



Graph 2. Inhibitory effect of ginkgo extract (0.2 g/l) on the tonus increase of the duodenal wall caused by different concentrations of acetylcholine (A = $6.6x10^{-9}$ mol/l; B = $2.2x10^{-8}$ mol/l; C = $6.6x10^{-8}$ mol/l i D = $2.2x10^{-7}$ mol/l)



Graph 3. Dose dependent tonus reduction of the ileal wall in the presence of ginkgo extract in different concentrations



Graph 4. Inhibitory effect of ginkgo extract (0.2 g/l) on the tonus increase of the ileal wall caused by different concentrations of acetylcholine (A = $6.6x10^{-9}$ mol/l; B = $2.2x10^{-8}$ mol/l; C = $6.6x10^{-8}$ mol/l i D = $2.2x10^{-7}$ mol/l)

DISCUSSION

Most researchers focused their studies on the effects of the ginkgo extract in the central nervous system. Starting from the fact that more than 60% of the extract is absorbed in the stomach and small intestine (Shultz *et al*., 2001), we decided to look upon the effects of the ginkgo extract on the pendular movements of the duodenum and ileum.

Pendular movements are rhythmic contractions and relaxations of the longitudinal layer of smooth muscles in the gut wall. They predominantly emerge from spontaneous electrical activity of smooth muscle cells themselves. Moreover, the intensity of this activity is under the control of neurotransmitters secreted by pacemaker neurons of the Meisner's plexus, which is a part of the enteric nervous system placed in the gut submucosa (Costa *et al*., 1982). Its most abundant neurotransmitter is acetylcholine, meaning that the motility and secretion of the gastrointestinal tract (GIT) are predominantly controlled by cholinegic neurons (Beleslin *et al*., 1978; Grider, 1989).

Acetylcholine exerts its stimulatory effects in the gastrointestinal tract through nicotinic receptors in ganglions and muscarinic receptors localized in nerve fibers and smooth muscle cell membranes. In the membranes of smooth muscle and secreting cells of the GIT the most abundant receptor subtype is M3 (Goyal, 1989). There are different data in literature about the subtypes of receptors present in presynaptic neural termini, where they regulate the release of endogenous ACh. Kilbinger *et al*. (1984) found receptors of the M2, and Radenković *et al*. (1998) of the M1 subtypes.

Atropine and scopolamine, tropoic alkaloids belonging to the group of cholinergic medicants, act as ACh antagonists on muscarinic receptors (Varagić, 2002). It is possible to assume that constituents of the ginkgo extract, act as competitive inhibitors of a certain type of muscarinic receptors, flavonoids being the most likely candidates because of their abundance and structural similarities to tropoic alkaloids. In support of this assumption, our data clearly show that ginkgo extract exerts dose dependent acute effects on the gut motility. It strongly reduces the tonus of the smooth muscles of the intestinal wall, no matter whether it was caused by their spontaneous activity, or ACh stimulated activity.

The inhibitory effect of the ginkgo extract was stronger in the ileum compared to duodenum. This can be explained by the differences in the presence and affinity of muscarinic receptors of different subtypes. Accordingly, Ruoff *et al*. (1991) found that muscarinic receptors of all 3 subtypes (M1, M2 and M3) were present in the different patterns along the rabbit gut, and their specific blockers exerted different effects in various parts of the intestinal canal.

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UTICAJ FARMAKOLOŠKI AKTIVNIH JEDINJENJA IZ EKSTRAKTA *GINKGO BILOBAE* NA KONTRAKTILNOST DUODENUMA I ILEUMA KUNIĆA

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SADRŽAJ

Biohemijska saznanja o prisustvu farmakološki aktivnih jedinjenja iz ekstrakta *Ginkgo bilobae*, dala su nov podstrek za izučavanje njihovog dejstva na receptore autonomnog nervnog sistema. Otuda je cilj našeg istraživanja bio da se ispita uticaj ekstrakta ove biljke na kontraktilnost glatkomišićnog sloja u zidu duodenuma i ileuma kunića metodom po Magnus-u. Rezultati istraživanja ukazuju da ispitivani ekstrakt izaziva dozno zavisno sniženje tonusa longitudinalne glatke muskulature duodenuma i ileuma, kao i da redukuje acetilholinom izazvanu stimulaciju spontane aktivnosti duodenuma i ileuma.