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STUDY REPORT

Study on cardiovascular and bronchial effects of

FLAVIN7® in isolated tissues

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STUDY LOCATED Department of Pharmacology and Pharmacotherapy
University of Debrecen.
Medical and Health Science Center
Address: H-4012 Debrecen. Nagyerdei krt. 98. POBox 12
Phone: + (36) 52 427-899
Fax: + (36) 52 427-899

SPONSOR: CRYSTAL INSTITUTE Ltd.
Address: H-3300 Eger, Telekessy u. 2.
Phone: + (36) 36-517688
Fax: + (36) 36-517644

INVESTIGATOR: András József Szentmiklósi, MD., PhD
Associate Professor

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Debrecen. June 10, 2003

András József Szentmiklósi, MD, PhD
Associate Professor
Investigator

Zoltán Szilvássy, MD, PhD, DSc
Director and Professor of Pharmacology

CONTENTS

1. AIM OF STUDY

The purpose of the present study was the examination of the action of FLAVIN7®, a fruit concentrate, on isolated atrial and ventricular tissues, as well as vascular and tracheal preparations.

The aim of this study was to examine

1/ the vasoactive properties of various concentrations of FLAVIN7® on abdominal aorta and carotid artery preparations of rabbits

2/ the endothelium-dependence of vasoactive effects of FLAVIN7®

3/ the action of various concentrations of FLAVIN7® on metacholine-precontracted tracheal preparations of rabbits

4/ the epithelium-dependence of FLAVIN7® in tracheal preparations

5/ the direct action of FLAVIN7® on sinu-atrial pacemaker activity, on contractility parameters (contractile force, + dP/dTmax, -dT/dTmax values, time to peak tension and half relaxation time) of electrically driven right papillary muscles of rabbits.

6/ the effect of various concentrations of FLAVIN7® on the vasoconstrictor activity of noradrenaline,

7/ the action of FLAVIN7® on the endothelium-dependent (metacholine) and endothelium-independent (nitroglycerine) relaxation of vascular tissue.

8/ the effect of FLAVIN7® on the adenosine-induced alterations of vascular and myocardial tissues

2. SUMMARY

The actions of a special fruit concentrate FLAVIN7® were analysed on isolated vascular and tracheal smooth muscle, as well as atrial and ventricular myocardium of rabbits and guinea pigs to assess the cardiovascular and bronchial activity of FLAVIN7® with special reference of its action on the endothelial functions and the involvement of adenosine mechanism.

In isolated, endothelium-intact aortic rings and carotid arteries of rabbits, FLAVIN7® induced a concentration-dependent relaxation of the phenylephrine-induced sustained contraction. The relaxant action of FLAVIN7® was more pronounced in carotid arteries than in aortic rings. In rings without functional endothelium, FLAVIN7® induced a concentration-dependent contraction.

FLAVIN7® (0.1 and 10 umol/l in gallic acid equivalents) enhanced the endothelium-dependent relaxation induced by low concentrations of metacholine, whereas antagonized the effects of higher levels of the muscarinic agonist. The fruit concentrate did not influence the endothelium-independent, nitroglycerine-induced relaxation.

The sensitivity of aortic smooth muscle preparations were reduced to noradrenaline (decrease of pD_2 values for noradrenaline and depression of maximum effects) in the presence of various concentrations of FLAVIN7® (3-10-30 umol/l).

In rabbit aortic tissues precontracted with phenylephrine, the relaxant actions of adenosine was significantly enhanced in the presence of FLAVIN7® indicating the sensitization of vascular adenosine receptors (containing A_2 adenosine receptors). In contrast, in electrically driven left atrial preparations (containing A_1 adenosine receptors), FLAVIN7® was capable of antagonizing the cardiodepressive actions of adenosine.

In rabbit tracheal preparations with intact epithelium, FLAVIN7® did not change the metacholine-induced sustained contraction, whereas in epithelium-denuded tracheal tissues exerted a concentration-dependent relaxation.

We have also shown that FLAVIN7® in spontaneously beating right atrial preparations and electrically stimulated ventricular papillary muscles virtually does

not influence the sinu-atrial pacemaker activity and the conventional contractility parameters of ventricular myocardium of rabbits (contractile force, maximum velocity of contraction and relaxation, time to peak tension, half relaxation time).

It can be concluded that FLAVIN7® can be a promising product in cardiovascular and respiratory pharmacology, but a number of *in vivo* experiments are needed to test its effectiveness in atherosclerosis and experimental bronchial asthma.

3. MATERIALS AND METHODS

3.1 Animals

Male, New Zeeland White rabbits were used in the study (weight: 3.2-3.8 kg). The experimental protocol has been approved by the Animal Care and Use Committee of University of Debrecen. The animals were kept in metal cages at room temperature and 12-h light cycle. The animals has access to standard granulated food with the addition of fresh vegetables.

3.2 Experimental protocol

3.2.1 Isolated heart tissues: spontaneously beating right atrial preparations and electrically driven right ventricular papillary muscles

Male, New Zeeland White rabbits (weight: 3.2-3.8 kg) were anaesthetized by injecting pentobarbitone sodium (30 mg/kg) through the marginal ear vein. Male, Heartley guinea pigs were sacrified by a blow on the head under light ether anaesthesia. Then the chest was opened and the heart was quickly excised and washed in oxygenated Krebs solution at 28^{o}C. Right atria (rabbits), left atria (guinea pigs) and right ventricular papillary muscles (rabbits) were isolated and set up in 10 ml vertical organ baths (TSZ-04, Experimetria, Budapest) containing Krebs solution (30^{o}C), the composition of which was (mmol/l): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; NaH_2PO_4, 1.0; $MgCl_2$, 1.2; $NaHCO_3$, 24.9 and glucose 11.5 with pH 7.4, when gassed with 95% O_2 and 5% CO_2. The papillary muscles of rabbits and left atria of guinea pigs were electrically paced (3 Hz, 1 ms, twice the threshold voltage) by a programmable stimulator (PST-02, Experimetria, Budapest). Isometric contractions of papillary muscles, left atria and spontaneously beating right atrial preparations were measured by means of a transducer (SD-01, Experimetria, Budapest), recorded by a polygraph (WR 3101, HSE, Hugstetten). Preparations were allowed to equilibrate for 60 min under an initial resting tension of 10 mN. After stabilization of the contractile parameters or sinu-atrial pacemaker activity, a cumulative concentration-response curve (E/[A]) was

generated with the test agent. When necessary, after exposure of the muscles to the agents (e.g. adenosine), preparations were washed with fresh Krebs solution until the recovery of initial parameters reached a steady level. Then FLAVIN7®, at various concentrations, was added to the nutrient solution. The contact time for this drug was 40 min before recording the subsequent E/[A] curves with an agent.

3.2.2 Isolated vascular and tracheal tissues

After anaesthetizing the animals, the abdominal aortae, the trachea and the common carotid arteries were removed and excess connective tissue excised. The vascular specimens were cut into rings approximately 4 mm long. Special care was taken to avoid contact with the luminal surface of the rings in order to preserve the endothelium. The endothelium was removed in some rings, where the luminal surface of the vessels was rubbed for 10-20 s with a wooden stick to damage the endothelium. The rings were vertically mounted on two stainless steel triangular clips, the lower clip being attached to a moveable support and the upper clip to a force displacement transducer in a 10-ml organ bath containing the physiological salt solution described above. The solution was maintained at 37^{o}C and continuously gassed with 95% oxygen and 5 % carbon dioxide, giving a pH 7.4. Tension of carotid and aortic vascular strips was measured isometrically by transducers (SG-01D, Experimetria) and the ouput fed to potentiometric recorders (SP-K2V, Riken Denshi). The specimens were given an initial tension of 20 mN and allowed to stabilize at this level of tension for at least 2 h. Following the equilibration period, preparations were exposed to 0.3 umol/l phenylephrine until a steady level of tension was reached. When the contraction was stable, cumulative administration of test agents (metacholine, adenosine, nitroglycerine, FLAVIN7®, etc.) were applied to the organ bath and were allowed to produce a maximal response. The tissues were then washed several times with fresh Krebs solution and allowed to recover. FLAVIN7®, at various concentrations, was applied under the resting state for 40 min and tissues then recontracted to the

control level of tone with phenylephrine. Then taking the concentration-response curves with the test agent was repeated.

Integrity or removal of endothelium was monitored functionally, at start of the experiments, by the quality of responses to metacholine (0.1 – 1 umol/l).

In another series of experiments, the neck of rabbits was opened and the trachea was carefully dissected and washed in fresh Krebs solution. Following this, circular segments (rings cut open, 6 mm in length, epithelium is not removed, unless otherwise specified) were prepared and mounted vertically in an organ bath (10 ml capacity) containing oxigenated Krebs solution (37°C, pH 7.4). Tissues were mounted under an initial resting tension of 10 mN and left to equilibrate for a period of 1 h before starting the experimental protocol. Tension changes were recorded using isometric force transducers (SG-01D, Experimetria) connected to a potentiometriac recorder (SP-K2V, Riken Denshi; OH-850, Radelkis). The concentration-dependent contractile responses were examined in tracheal preparations (epithelium-intact and epithelium-denuded) precontracted with metacholine (0.3 umol/l metacholine).

3.3 Statistical analysis

Agonist-induced change in mechanical activity was expressed as the percentage reduction of pre-drug contractile force. Individual E/[A] curve data were fitted by means of a least-square iterative computer program to a logistic function of the form: $E=E_{max}[A]^{n}_{H}/[A]^{n}_{H}+[EC_{50}]^{n}_{H}$, where E denotes the effect, E_{max} is the asymptote, [A] is the concentration of the agonist, EC_{50} is the concentration producing a half-maximal response, and n_H is the midpoint slope parameter.

For evaluating the sensitizing and antagonistic actions of the compounds pA25 values (concentrations inducing 25% decrease of pre-drug contractile force) were determined by linear regression analysis.

The data are expressed as means ± S.E.M. The EC_{50} values are expressed as their negative base 10 logarithms (pD_2 values) throughout the text. Multiple comparisons between the experimental groups were performed by one way analysis of variance (ANOVA) with a Newman-Keuls post hoc test. Statistical significance was

evaluated also by the Student's *t*-test, where appropriate; $P < 0.05$ was taken as the level of significance.

In the figures points are the mean values of the experiments and bars represent S.E.M.

Materials

The following drugs were used: adenosine, metacholine, nitroglycerine, papaverine hydrochloride (Sigma, St. Louis). Concentrated FLAVIN7® was supplied by CRYSTAL INSTITUTE Ltd. The molar concentrations of FLAVIN7® were expressed as gallic acid equivalents.

4. RESULTS

4.1. Action of FLAVIN7® on abdominal aorta of rabbits precontracted with phenylephrine: study on endothelium dependence

In isolated rings of rabbit abdominal aorta precontracted with phenylephrine (0.3 umol/l), FLAVIN7® exerted a concentration-dependent response. In endothelium-intact preparations, FLAVIN7® induced a concentration-related relaxation (Fig. 1and 2, Table 1) with a –log EC25 value of 4.98 ± 0.19 (n = 6). It should be noted that FLAVIN7® at a concentration of 100 umol/l, exerted a biphasic effect, an initial, slight relaxation followed by a strong contraction (Fig. 2 and Table 2). This contraction exceded the phenylephrine-induced precontraction by 49 ± 3 % (n = 6). Rubbed rings displayed a pronounced dose-dependent contractions (Fig. 3 and 4) with –logEC25 value of 4.59 ± 0.15 (n = 6).

4.2. Action of FLAVIN7® on carotid arteries of rabbits precontracted with phenylephrine: study on endothelium dependence

In isolated rings of rabbit carotid arteries precontracted with phenylephrine (0.3 umol/l), FLAVIN7® exerted a concentration-dependent response. In endothelium-intact preparations, FLAVIN7® induced a concentration-related relaxation (Fig. 5 and 6, Table 3) with a –log EC25 value of 5.61 ± 0.17 (n = 6). In carotid arteries, FLAVIN7® at a concentration of 100 umol/l displayed, like abdominal aorta, strong vasoconstrictor actions. (Fig. 6) Rubbed rings of carotid arteries, like aorta, exerted pronounced dose-dependent contractions (Fig. 7 and 8, Table 4) with –logEC25 value of 4.65 ± 0.05 (n = 6).

4.3. Effect of FLAVIN7® on epithelium-intact and rubbed preparations of rabbit trachea

In rabbit tracheal preparations with intact epithelium (n = 9) FLAVIN7® at concentrations of 0.1 – 100 umol/l did not influence significantly the tone of tracheal smooth muscle precontracted with metacholine (Fig. 9 and Table 5). In contrast, in epithelium-denuded tracheal tissues (n = 7), FLAVIN7® was able to induce a concentration-dependent relaxation (Fig. 10 and Table 6).

4.4. Effect of various concentrations of FLAVIN7® on noradrenaline-induced contractions in rabbit abdominal aorta

In rabbit aorta preparations, noradrenaline (10 pmol/l – 30 umol/l) induced a concentration-dependent contraction with a pD_2 value of 6.81 ± 0.05 (n = 8). In the presence of 3 umol/l FLAVIN 7, concentration-response curves for noradrenaline were shifted to the right with a pD_2 value of 6.02 ± 0.11 (n = 6; significantly different from control: $p<0.05$) with depression of the maximum response (Fig. 11; Table 7 and 8). 10 umol/l FLAVIN7® caused a further rightward shift in the noradrenaline dose-response curves with a depressed maximum response (n = 4; pD_2 5.77 ± 0.27; Fig 11; Table 9). FLAVIN7® at a concentration of 30 umol/l did not evoke further reduction in the contractile action of noradrenaline (n = 6; pD_2 5.76 ± 0.09; Fig. 11 and Table 10).

4.5. Effect of various concentrations of FLAVIN7® on sinu-atrial pacemaker activity of right atrial myocardium of rabbits.

In control conditions, the sinu-atrial rate of spontaneously beating right atria of rabbits was 149 ± 7 (n = 7). FLAVIN7® at a wide range of concentrations (0.1 – 100 umol/l as gallic acid equivalents) did not influence significantly the sinu-atrial rate (Fig. 12 and Table 11).

4.6. Action of FLAVIN7® on contractility parameters of electrically stimulated right ventricular papillary muscles of rabbits.

In this series of experiments, contractile force, maximum velocity of contraction (+dP/dTmax), maximum velocity of relaxation (-dP/dTmax), time to peak tension (TPT) and half relaxation time (HRT) were estimated in electrically driven rabbit papillary

muscles (n = 7). Contractile force slightly decreased at higher concentrations (3, 30 and 100 umol/l) of FLAVIN7®, but these changes have no biological significance (Fig. 13 and Table 12). After flavonoid exposition, at lower concentrations (0.1-0.3 umol/l), a slight increase in +dP/dTmax was revealed, whereas at 100 umol/l concentration a moderate decrease was observed without physiological significance (Fig. 14 and Table 13). Similar results were also found for maximum velocity of relaxation (Fig. 15 and Table 14). There were alterations neither in time to peak tension (TPT), nor half relaxation time (HRT) of papillary muscles after incubation of various doses of FLAVIN7® (Fig 16 and 17; Table 15 and 16).

4.7. Action of FLAVIN7® on endothelium-dependent, metacholine-induced relaxation in abdominal aortic rings of rabbits.

In aortic rings with functional endothelium, metacholine, a stable muscarinic receptor agonist (10 pmol/l – 3 umol/l) induced a concentration-dependent relaxation of the sustained contractions induced by phenylephrine (Fig. 18 and 19). In the presence of 0.1 umol/l FLAVIN7®, the relaxant actions of lower concentrations of metacholine (10 pmol/l – 0.1 umol/l) were enhanced compared to control, whereas the vasodilator effect of higher concentrations (1-3 umol/l) of metacholine was moderately reduced (Fig. 18; Table 17 and 18). Similar results were found also in the presence of 10 umol/l FLAVIN7® (Fig. 19; Table 17 an 19).

4.8. Action of FLAVIN7® on endothelium-independent, nitroglycerine-induced relaxation in abdominal aortic rings of rabbits.

In phenylephrine-precontracted rabbit aortic tissues with functional endothelium, nitroglycerine in a wide concentration range (10^{-14} – 10^{-6} mol/l) exerted a dose-dependent relaxation (n = 5). FLAVIN7® modified the vasorelaxant actions of nitroglycerine (Fig. 20 and 21; Table 20-22) in neither low (0.1 umol/l), nor higher (10 umol/l) concentrations.

4.9. Action of FLAVIN7® on adenosine-induced relaxation in abdominal aortic rings of rabbits.

In rabbit aortic preparations with intact endothelial layer, adenosine (0.1 umol/l – 1 mmol/l) induced concentration-dependent relaxation (Fig. 22 and 23). FLAVIN7® (1 umol/l) virtually did not influence the vasorelaxant action of adenosine, although the action of 0.1 and 0.3 umol/l adenosine was slightly potentiated (n = 9; Fig. 22 ; Table 23 and 24). FLAVIN7® at higher concentration (10 umol/l), however, significantly enhanced the relaxation induced by the purine nucleoside (n = 7; Fig. 23; Table 25 and 26).

4.10. Action of FLAVIN7® on adenosine-induced decrease of mechanical activity of electrically stimulated left atrial preparations of guinea pigs.

Adenosine evoked a concentration-dependent decrease of contractile force in atrial myocardium (Fig. 24). FLAVIN7® at concentrations of 1, 10 and 100 umol/l, antagonized the myocardial actions of adenosine Fig. 24., Tables 27-30). As shown in Fig. 24, FLAVIN7® at various concentrations was capable of inducing parallel rightward shifts in the concentration-response curves for adenosine without altering the maximum responses (pD_2 values for control: 5.71 ± 0.04; at 1 umol/l FLAVIN7®: 5.47 ± 0.04; at 10 umol/l FLAVIN7® 5.39 ± 0.11; at 100 umol/l FLAVIN7® 4.83 ± 0.14.

As FLAVIN7® is a not a single compound, therefore Schild regression analysis was not performed.

5. DISCUSSION

Recently, there has been a great deal of interest in polyphenolic compounds due to their beneficial cardiovascular and pulmonary actions of these compounds both in animals and humans. Polyphenols are present in all plants. There are more than 8000 phenolic structures that have been identified. More than 10 classes of polyphenols have been defined on the basis of chemical structure. The most common polyphenolic compounds are the flavonoids. Flavonoids can be categorized into 13 classes comprising more than 5000 compounds. The most common flavonoids are flavones, flavonols, and their glycosides (Bravo, 1998). The majority of polyphenols in wine are phenolic acids, anthocyanins, tannins, and other flavonoids. The most abundant phenolic compound in fruits is flavonol. The predominant flavonoid in onions is quercetin glycoside, whereas in tea and apples it is quercetin-3-rutinoside (Bravo, 1998).

A number of studies have described that an inverse association exists between flavonoid intake and risk of coronary disease(Hertog et al., 1993; 1995; Knekt et al., 1996; Yochum et al., 1999). It has been found that a high intake of flavonoids (approximately 30 mg/day) was associated with approximately a 50% reduction in the mortality rate of coronary heart disease compared with patients who had a low flavonoid intake (less than 19 mg/day) (Hertog et al, 1993). In another study (Knekt et al., 1996), it was described that onions and apples, rich sources of dietary flavonoids, were associated with a reduction in coronary mortality. On the basis of most of the relevant studies, it has been accepted that flavonoids have a protective action against coronary mortality.

One of the main constituent of FLAVIN7® is a concentrated red grape extract. The red wine and red grape juice are abundant sources of polyphenolic compounds and more than 200 phenolic compounds have been identified in red wine (German and Walzem, 2000). Red wine has been reported to inhibit oxidation of LDL in vitro

(Frankel et al., 1993; Kerry and Abbey, 1997) and it increases antioxidant capacity of plasma (Duthie et al., 1998). The antioxidant capacity of red wine can be due to phenolic acids, flavonols, monomeric catechins, and polymeric anthocyanidins. It is estimated that red wine contains about 30 mg/l of flavonols (quercetin and kaempferol) and 140 mg/l of phenolic acids. In addition, phenolic compounds have antithrombotic effects that seems to be the result of decreased susceptibility of platelet aggregation, reduced synthesis of prothrombotic mediators, decreased expression of adhesion molecules, and tissue factors (see Rotondo and de Gaetano for a review, 2000). Wine polyphenols have been shown to modulate the NO production of the vascular endothelium, resulting in vasorelaxation (Rotondo and de Gaetano, 2000). Among the vasoactive compounds of red wine, quercetin and resveratrol have of great significance. There is evidence that foods providing 16 to 24 mg/day of quercetin have protective actions against cardiovascular diseases (Hertog et al., 1993; 1995; Knekt et al., 1996). Chung et al. (1993) have shown that quercetin inhibits platelet aggregation in vitro, whereas Tzeng et al. (1991) have described that this compound is capable of reducing thromboxane synthesis in vivo.

Red wine is also a rich source of resveratrol and the cardioprotective effects associated with moderate consumption of wine can be due, at least in part, to this compound. There is evidence that resveratrol in vitro inhibits LDL oxidative susceptibility (Frankel et al. 1993), platelet aggregation (Pace-Asciak et al., 1995). It has antioxidant, free radical–scavenging activity and increases NO release (Hung et al., 2000). It appears that resveratrol may decrease risk of cardiovascular diseases by multiple mechanisms.

It has also been shown that the other constituents of FLAVIN7® including blackberry, heart-cherry, black currant, red currant, plum and apple contain various polyphenols that have beneficial physiological activity (Serraino et al., 2003; Klesk and Qian, 2003; Halvorsen et al., 2002; Jenkins et al., 2002; Hwang et al., 2001; Hetenyi and Valyi-Nagy, 1969; Nielsen et al., 2003; Erlund et al., 2003; Cacace and Mozza, 2002; Nakamura et al., 2002; Slimestad and Solheim, 2002), Maatta et al., 2001).

In this study, we have shown that FLAVIN7® exerts a pronounced action on the contractile activity of vascular tissues. In abdominal aortic preparations and carotid

arteries, FLAVIN7® induced a concentration-dependent relaxation when endothelium remained intact. It is interesting to note that vasorelaxant property of FLAVIN7® was more pronounced in the middle-size vessels (carotid arteries) than in the large vessel aorta. When this tendency is consistent, it can be supposed that FLAVIN7® is a potent vasodilator in the microcirculation. To establish this supposition, of course, studies on microvessels are needed.

In rings without functional endothelium, FLAVIN7® did not produce any relaxant effect, but induces a concentration-dependent contraction. On the basis of EC25 values, contractions were similar both in aortic and carotid artery preparations. These results demonstrate that some constituents of FLAVIN7® induce an endothelium-dependent relaxation. According to literature data, flavonoids are vasodilator compounds. Most of their actions are reported to be independent of the presence of functionally intact endothelium (Duarte et al., 1993; Herrera et al., 1996). There are flavonoids, the vasorelaxant effects of which are dependent on the integrity of endothelium, e.g. red wine polyphenolic compounds and leukocyanidol (Andriambeloson et al., 1997; Fitzpatrick et al., 1993), as well as dioclein (Lemos et al., 1999). Nakamura et al. (2002) have described that black current concentrate (a constituent of FLAVIN7®) is able to induce an endothelium-dependent vasorelaxation. This response could be abolished after endothelium removal, but no contractions are developed. The relaxations induced by black current concentrate have been shown to be mediated by H1 histamine receptors. In the case of FLAVIN7®, it can be supposed that this product contains some constituents, which are able to induce endothelium-dependent relaxation. As far as the FLAVIN7®-induced contraction are concerned, to our best knowledge, there are no data in the literature describing polyphenolic compounds evoking strong contractions in endothelium-denuded vessels. To elucidate this interesting action of FLAVIN7®, further laboratory experiments are necessary.

FLAVIN7®, at concentrations of 0.1 and 10 umol/l enhanced the endothelium-dependent relaxation of aortic preparations, at least in lower concentrations of metacholine. The relaxant actions of higher levels of metacholine was, however,were reduced. It is suggested that some constituents of FLAVIN7® can improve the NO production of endothelial cells, when muscarinic receptor stimulation is moderate. The reverse can be true, when „non-physiological” concentrations of metacholine has

been applied. Recently, Sanae and his coworkers (2002) have described that in rat aorta, gallic acid is able to inhibit endothelium-dependent relaxation probably by inactivation of NO. As FLAVIN7® has a number of constituents of various biological activity, therefore it can be presumed that at higher metacholine concentrations the inhibitory action of gallic acid (a constituent of FLAVIN7®) has been more effectively manifested.

We have examined the action of FLAVIN7® also on the endothelium-independent, nitroglycerine-induced relaxation, but the concentrate of fruits did not modify the actions of nitroglycerine indicating that vascular smooth muscle functions were not impaired in the presence of FLAVIN7®.

It was found that various concentrations of FLAVIN7® reduced the sensitivity of the aortic preparations to the contractile effect of noradrenaline. The pD_2 values for noradrenaline measured for FLAVIN7®-treated aortic tissues were significantly lower than corresponding values determined with control. Although, experiments in endothelium-denuded preparations were not carried out, it can be supposed that the reduced sensitivity of aortic rings to noradrenaline is due to the increased production of endothelial NO in the presence of FLAVIN7®. Our results are in a good agreement with the findings of Deckert et al. (2002), who described similar effects for the polyphenolic compounds of red wine in rat aortic preparations.

In this study, we have found that FLAVIN7® exerts an opposite action on vascular (A_2) and myocardial adenosine (A_1) receptors. Adenosine is an important endogenous substance released from apparently all cells, including heart and vessels. Adenosine has been known to act on specific P1 purinoceptors. The P1 receptor family comprises A_1, A_{2A}, A_{2B} and A_3 adenosine receptors, identified by biochemical, pharmacological and molecular biological studies. It is well established that and A_2 adenosine receptors are highly expressed in endothelium and vascular smooth muscle (Olsson and Pearson, 1990), whereas A_1 adenosine receptors are predominant in guinea pig atrial myocardium (Collis, 1983; Ford and Broadley, 1997). According to our recent findings, FLAVIN7® sensitized A_2 adenosine receptors to adenosine in rabbit aortic smooth muscle, whereas antagonized A_1 adenosine receptors in guinea pig atrial myocardium.

Interactions between polyphenolic compounds and adenosine receptors has recently been described. Flavone derivatives, such as galangin, were found to bind to three subtypes of adenosine receptors in the micromolar range. Galangin has Ki

values of 1 umol/l for both rat A_1 and A_{2A} adenosine receptors and 3 umol/l for human A_3 receptors (Karton et al., 1996). Genistein was found to bind A_1 and A_{2A} adenosine receptors (Jacobson et al., 2002). Flavonoids of Microtea debilis, were found to have adenosine antagonistic features in rats (Hasrat, 1997). From these compounds, cirsimarin was isolated as the active component and displayed potent A_1 adenosine receptor antagonist property. The effectiveness of Microtea debilis can probably be due to A_1 adenosine receptor antagonistic feature of cirsimarin (Hasrat, 1997). In addition, aurones and aurone derivatives, e.g. hispidol, sulfuretin, aureusidin and maritimetin have also been found as antagonists on A_1 and A_{2A} adenosine receptors (Jacobson et al., 2002).

As adenosine receptors are involved in the homeostasis of the immune, cardiovascular, respiratory and central nervous system, therefore any compound that has a modulatory action on adenosine receptors, can be potential drugs for treating various cardiovascular, respiratory and central nervous disorders.

FLAVIN7® has a characteristic property in purine pharmacology: some of its constituents enhances the action of adenosine on vascular adenosine receptors. Hypothetically, these effects could lead to vasodilation and could induce vasoprotective, cardioprotective, cerebroprotective and antiasthmatic action. To decide this suggestion, laboratory experiments are needed. At any rate, to our best knowledge, there are no reports in the literature in the adenosine receptor-sensitizing actions of flavonoids.

As for A_1 antagonistic properties of FLAVIN7® are concerned, this effect can have an impact in antagonizing A_1 adenosine receptors in a variety of cells similarly to caffeine or theophylline.

We have shown that in acute experiments, FLAVIN7® in spontaneously beating right atrial preparations and electrically stimulated ventricular papillary muscles virtually does not influence the sinu-atrial pacemaker activity and the conventional contractility parameters of ventricular myocardium of rabbits (contractile force, maximum velocity of contraction and relaxation, time to peak tension, half relaxation time).

FLAVIN7® did not modify the tone of metacholine-precontracted tracheal preparations with intact epithelium, whereas it exerted a moderate relaxant effect after damage of tracheal epithelium. As in bronchial asthma, because of various inflammatory mediators, denudation of epithelial layer can be occurred, therefore this

finding can suggest to study the action of FLAVIN7® in experimental bronchial asthma.

On the basis of our results and literature data for polyphenolic compounds, we suggest to perform a careful and detailed study on the possible antiatherogenic and antiasthmatic action of FLAVIN7® or its constituents.

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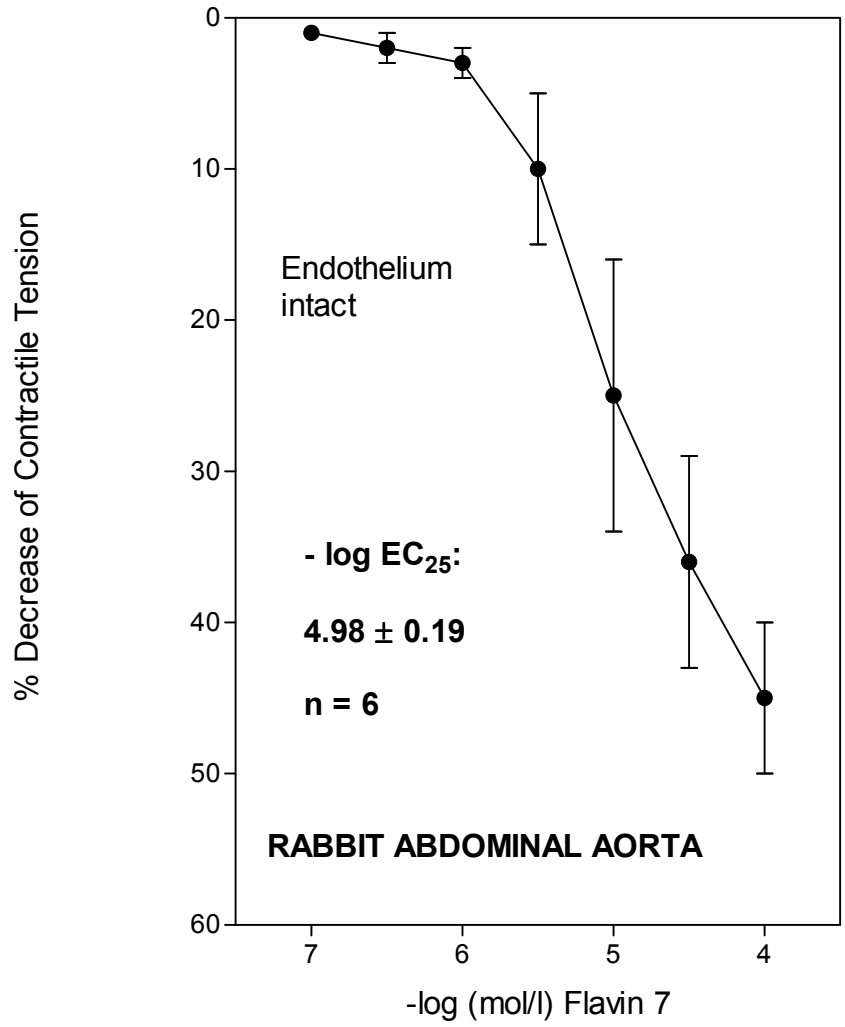

Fig 1. Relaxant action of FLAVIN7® on rabbit abdominal aorta rings precontracted with phenylephrine

Fig.2 Effect of FLAVIN7® on rabbit abdominal aortic ring (endothelium intact): original record

PHE: phenylephrine 0.3 umol/l, PAP: papaverine 100 umol/l

Numbers indicate the negative molar logarithm of FLAVIN7®

Table 1

Relaxant effect of FLAVIN7® on rabbit abdominal aorta (endothelium intact) precontracted with phenylephrine

	relaxation in % of precontraction after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) FLAVIN7®						
	0,0	0,0	0,0	17,0	30,0	41,0	46,0
	2,0	4,0	4,0	8,0	12,0	38,0	42,0
	2,0	4,0	7,0	29,0	66,0	66,0	27,0
	1,0	2,0	2,0	3,0	9,0	20,0	36,0
	0,0	0,0	0,0	0,0	8,0	18,0	62,0
	0,0	4,0	4,0	4,0	23,0	32,0	54,0
Mean	0.8	2	3	10	25	36	45
S.E.M.	0.4	1	1	5	9	7	5

- log EC25 4.98 ± 0.19

n = 6

Molar concentrations of FLAVIN7® are expressed as gallic acid equivalents

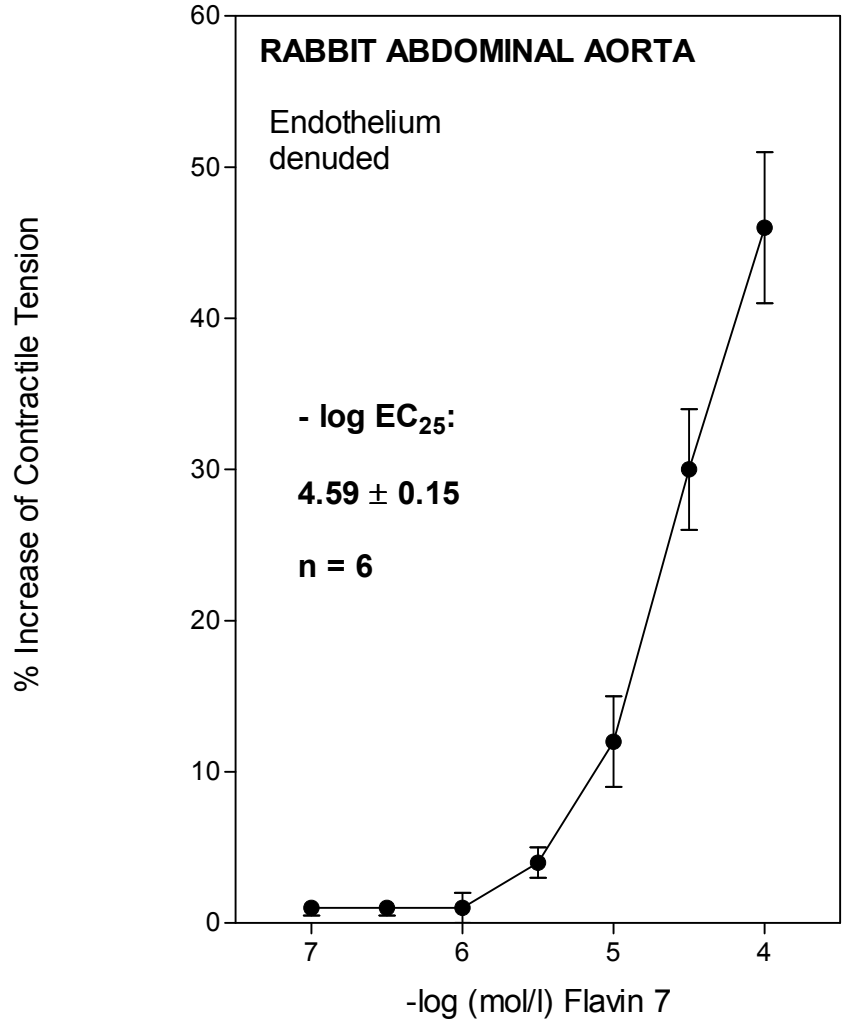

Fig 3 Vasoconstrictor action of FLAVIN7® on rabbit abdominal aortic rings (endothelium denuded) precontracted with phenylephrine

Fig.4 Effect of FLAVIN7® on rabbit abdominal aortic ring precontracted with phenylephrine (endothelium denuded): original record
PHE: phenylephrine 0.3 umol/l, PAP: papaverine 100 umol/l
Numbers indicate the negative molar logarithm of FLAVIN7®

Table 2

Vasoconstrictor effect of FLAVIN7® on rabbit abdominal aorta (endothelium denuded) precontracted with phenylephrine

	contraction in % of precontraction after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) FLAVIN7®						
	0,0	0,0	0,0	2,0	4,0	10	22,0
	0,0	0,0	0,0	2,0	10,0	28,0	44,0
	0,0	0,0	1,0	6,0	20,0	39,0	54,0
	0,0	0,0	0,0	4,0	14,0	33,0	53,0
	3,0	3,0	6,0	10,0	19,0	38,0	53,0
	1,0	1,0	1,0	2,0	7,0	32,0	48,0
Mean	0.7	0,7	1	4	12	30	46
S.E.M.	0.5	0,5	1	1	3	4	5

- log EC25: 4.59 ± 0.15

n = 6

Molar concentrations of FLAVIN7® are expressed as gallic acid equivalents

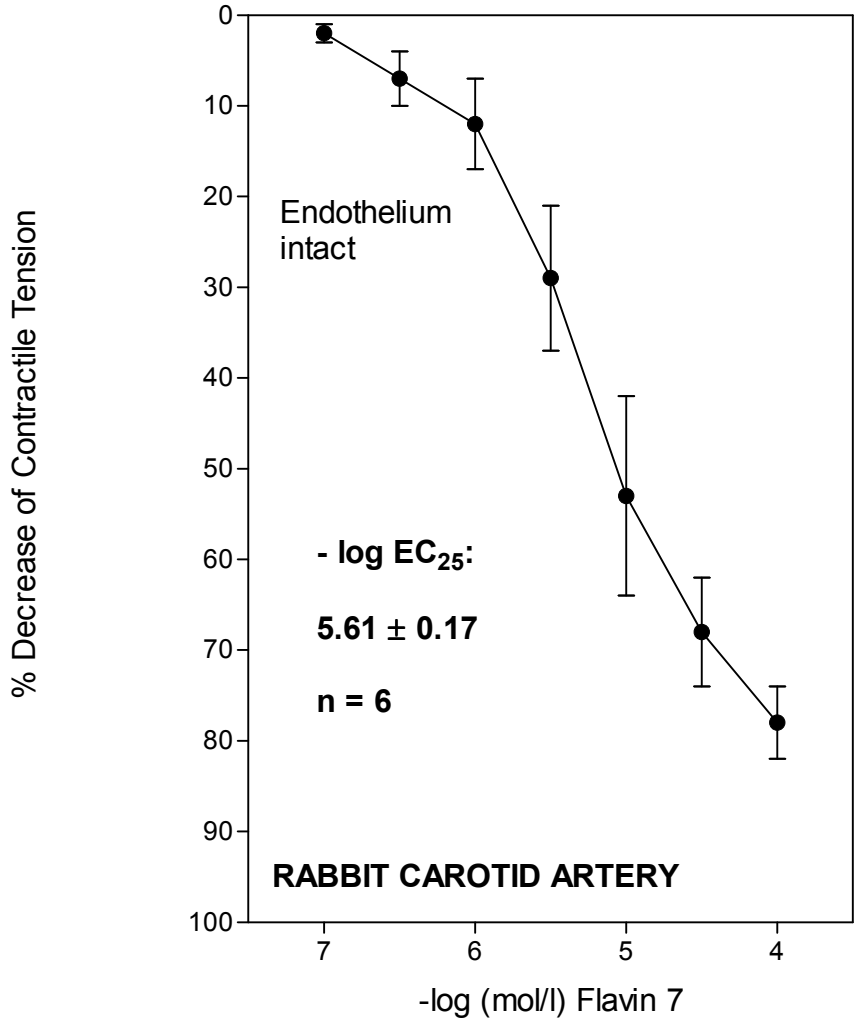

Fig. 5 Relaxant action of FLAVIN7® on rabbit carotid artery precontracted with phenylephrine

Fig.6 Effect of FLAVIN7® on rabbit carotid artery (endothelium intact): original record

PHE: phenylephrine 0.3 umol/l, PAP: papaverine 100 umol/l

Numbers indicate the negative molar logarithm of FLAVIN7®

Table 3

Relaxant effect of FLAVIN7® on rabbit carotid artery (endothelium intact) precontracted with phenylephrine

	relaxation in % of precontraction after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) FLAVIN7®						
	0,0	0,0	0,0	13,0	33,0	61,0	81,0
	12,0	19,0	31,0	63,0	100,0	-	-
	2,0	4,0	5,0	36,0	64,0	89,0	91,0
	3,0	8,0	15,0	28,0	48,0	72,0	82,0
	4,0	12,0	20,0	32,0	40,0	64,0	70,0
	0,0	0,0	3,0	5,0	32,0	56,0	68,0
Mean	2	7	12	29	53	68	78
S.E.M.	1	3	5	8	11	6	4

- log EC25 5.61 ± 0.17

n = 6

Molar concentrations of FLAVIN7® are expressed as gallic acid equivalents

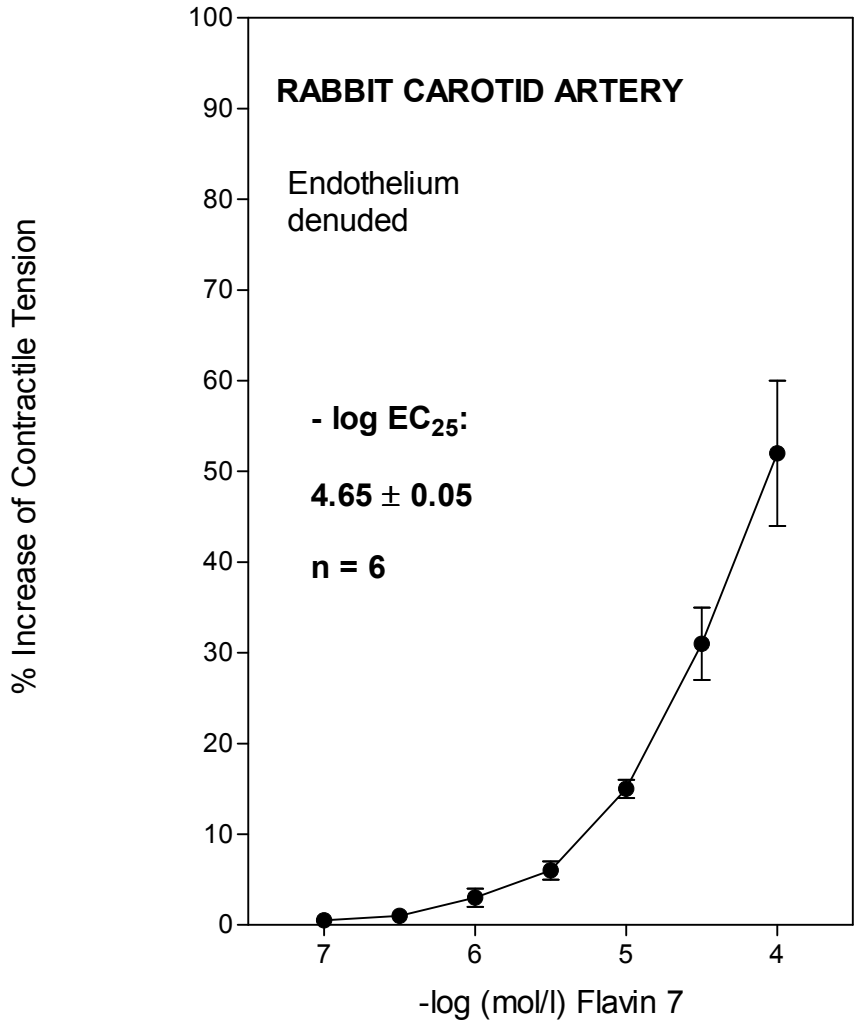

Fig. 7 Vasoconstrictor action of FLAVIN7® on rabbit carotid artery precontracted with phenylephrine

Fig. 8. Effect of FLAVIN7® on rabbit carotid artery (endothelium denuded): original record

PHE: phenylephrine 0.3 umol/l, PAP: papaverine 100 umol/l

Numbers indicate the negative molar logarithm of FLAVIN7®

Table 4

Vasoconstrictor effect of FLAVIN7® on rabbit carotid artery (endothelium denuded) precontracted with noradrenaline

	contraction in % of precontraction after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) FLAVIN7®						
	2,0	2,0	3,0	7,0	14,0	29	56,0
	0,0	0,0	0,0	6,0	19,0	25,0	44,0
	0,0	3,0	5,0	8,0	14,0	21,0	36,0
	1,0	2,0	4,0	8,0	16,0	28,0	42,0
	0,0	0,0	0,0	0,0	13,0	50,0	88,0
	0,0	2,0	6,0	8,0	15,0	32,0	48,0
Mean	0.5	1	3	6	15	31	52
S.E.M.	0.3	0,4	1	1	1	4	8

- log EC25 4.65 ± 0.05

n = 6

Molar concentrations of FLAVIN7® are expressed as gallic acid equivalents

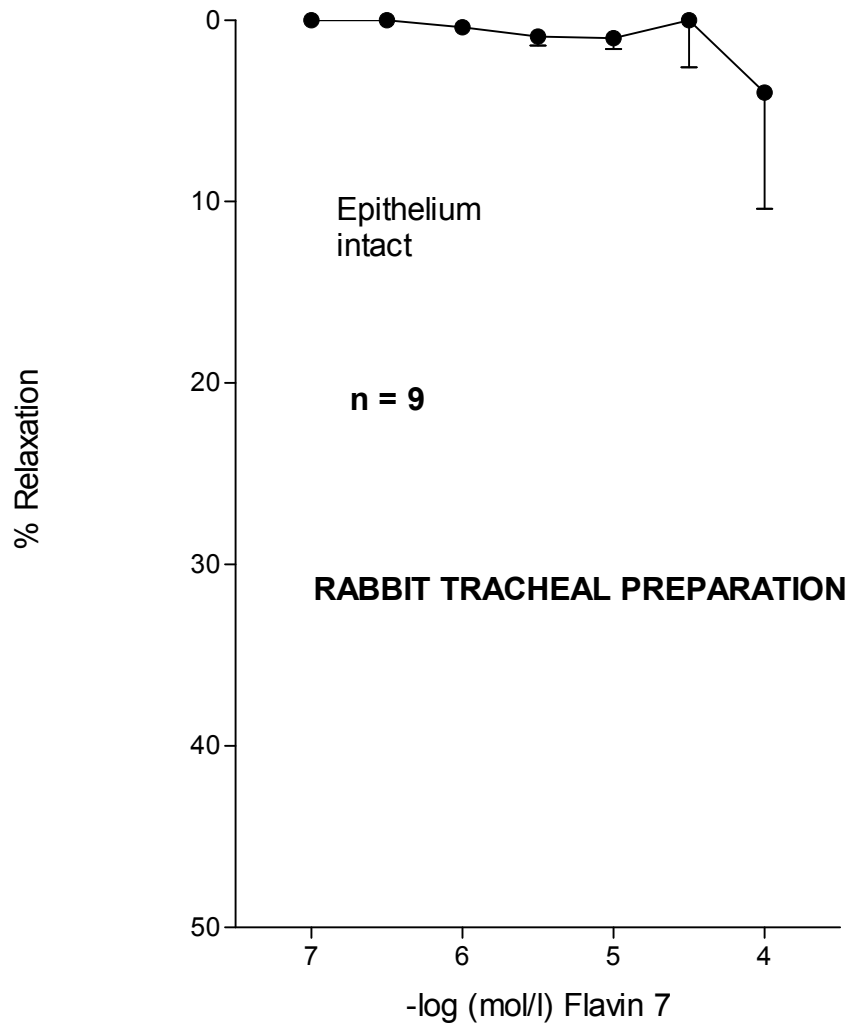

Fig. 9 Effect of FLAVIN7® on rabbit tracheal preparations (epithelium intact) precontracted with metacholine

Table 5

Effect of FLAVIN7® on the contractile tension of rabbit tracheal smooth muscle (epithelium intact)

% decrease of contraction after exposure to						
7	6.5	6	5.5	5	4.5	4
-log (mol/l) FLAVIN7®						
0	0	0	0	0	0	0
0	0	2	2	2	+4	+4
0	0	0	0	0	0	0
0	0	0	0	0	0	9
0	0	2	4	4	6	18
0	0	0	0	0	7	33
0	0	0	0	0	+19	+38
0	0	0	2	4	5	12
0	0	0	0	2	4	8

Mean	0	0	0.4	0.9	1	+0.1	4
S.E.M.	0	0	0.3	0.5	0.6	2.6	6.4

n = 9

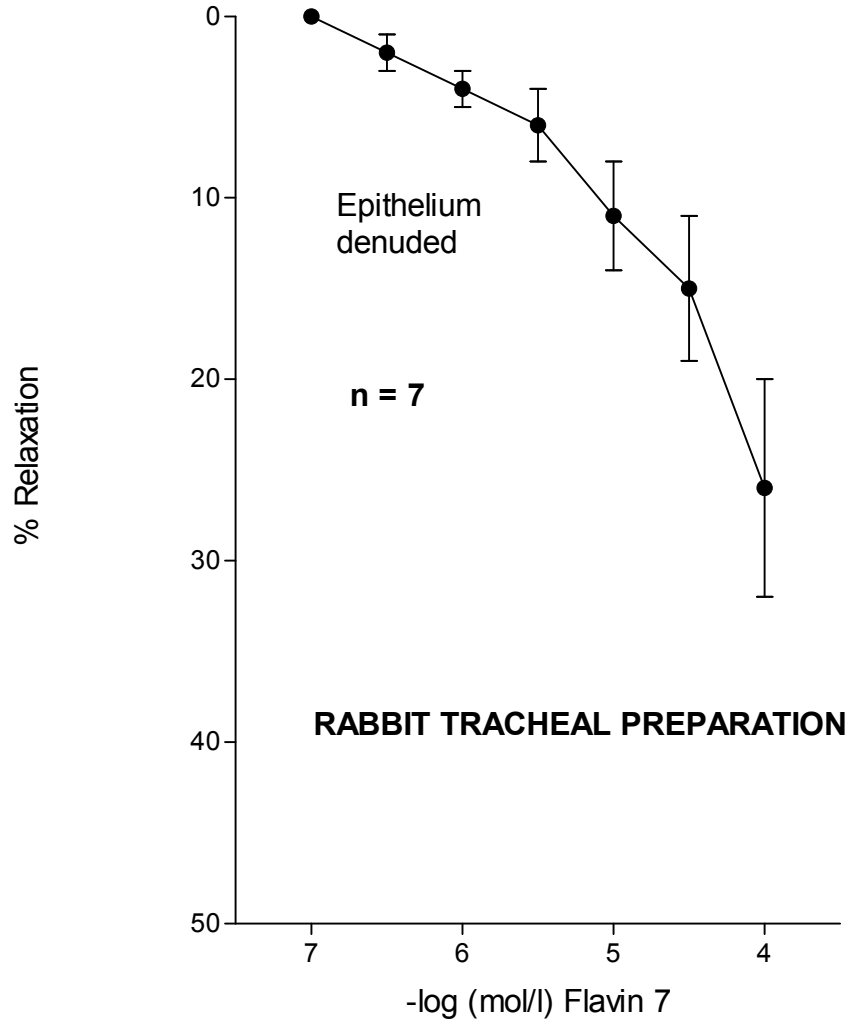

Fig. 10 Effect of FLAVIN7® on rabbit tracheal preparations (epithelium denuded) precontracted with metacholine

Table 6

Effect of FLAVIN7® on the contractile tension of rabbit tracheal smooth muscle (epithelium denuded)

% decrease of contraction after exposure to						
7	6.5	6	5.5	5	4.5	4
-log (mol/l) FLAVIN7®						
0	0	0	0	0	0	0
0	0	2	4	8	17	33
0	2	4	8	18	28	43
0	2	6	6	6	6	12
0	0	0	0	7	14	36
0	0	4	8	16	16	37
0	8	10	15	23	23	23

Mean	0	2	4	6	11	15	26
S.E.M.	0	1	1	2	3	4	6

n = 7

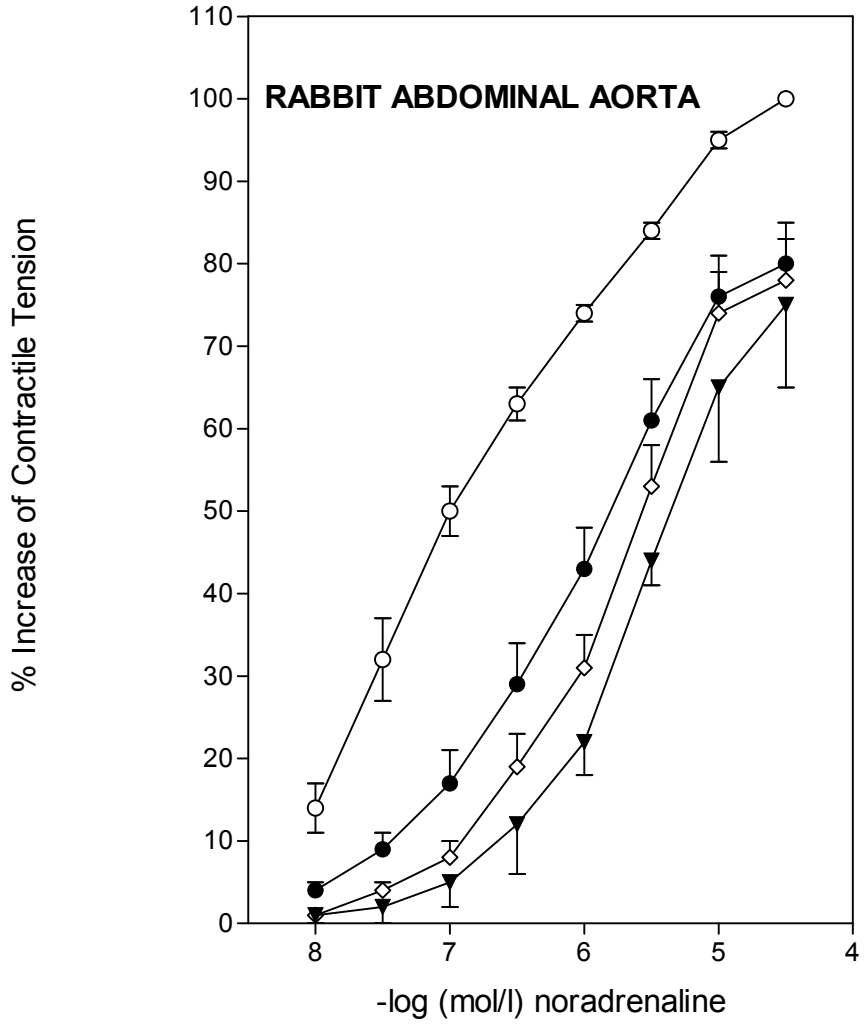

Fig.11 Effect of various concentrations of FLAVIN7® on the concentration-response curves for noradrenaline in endothelium-intact abdominal aortic rings of rabbits.

○ in the absence of FLAVIN7® (n = 8)

● in the presence of 3 umol/l FLAVIN7® (n = 6)

◊ in the presence of 10 umol/l FLAVIN7® (n = 4)

▼ in the presence of 30 umol/l FLAVIN7® (n = 6)

Values are expressed as % of control maximum response.

Table 7

Effect of FLAVIN7® on the noradrenaline-induced increase of contractile tension of rabbit abdominal aorta (Control)

% increase of contraction after exposure to							
8	7.5	7	6.5	6	5.5	5	4.5
- log (mol/l) noradrenaline							
in the absence of FLAVIN7® (Control)							
5,0	19,0	46,0	67,0	79,0	87,0	93,0	100,0
23,0	44,0	59,0	67,0	75,0	83,0	97,0	100,0
16,0	40,0	54,0	62,0	71,0	79,0	95,0	100,0
24,0	43,0	56,0	64,0	73,0	82,0	96,0	100,0
25,0	46,0	58,0	64,0	73,0	83,0	96,0	100,0
13,0	28,0	48,0	60,0	75,0	85,0	93,0	100,0
5,0	16,0	37,0	53,0	68,0	85,0	95,0	100,0

	4,0	17,0	44,0	63,0	80,0	89,0	94,0	100,0
Mean	14	32	50	63	74	84	95	100
S.E.M.	3	5	3	2	1	1	0,5	0

pD_2: 6.81 ± 0.05

E_{max}: 105 ± 2

n = 8

Table 8

Effect of 3 umol/l FLAVIN7® on the noradrenaline-induced increase of contractile tension of rabbit abdominal aorta

% increase of contraction after exposure to							
8	7.5	7	6.5	6	5.5	5	4.5
- log (mol/l) noradrenaline							
in the presence of FLAVIN7® (3 umol/l)							
0,0	1,0	5,0	13,0	35,0	63,0	83,0	87,0
4,0	10,0	16,0	26,0	36,0	49,0	62,0	66,0
8,0	15,0	32,0	44,0	57,0	72,0	89,0	94,0
5,0	10,0	19,0	30,0	41,0	65,0	70,0	74,0
3,0	5,0	9,0	17,0	29,0	45,0	67,0	72,0
2,0	10,0	21,0	41,0	60,0	74,0	86,0	88,0
Mean 4	9	17	29	43	61	76	80

S.E.M.	1	2	4	5	5	5	5	4

$pD_{2:}$ 6.02 ± 0.11*

$E_{max:}$ 89 ± 4*

n = 6

* values are significantly ($p<0.05$) different from control

Table 9

Effect of 10 umol/l FLAVIN7® on the noradrenaline-induced increase of contractile tension of rabbit abdominal aorta

	% increase of contraction after exposure to							
	8	7.5	7	6.5	6	5.5	5	4.5
	- log (mol/l) noradrenaline							
	in the presence of FLAVIN7® (10 umol/l)							
	0,0	0,0	0,0	2,0	11,0	37,0	66,0	78,0
	0,0	2,0	4,0	10,0	21,0	47,0	74,0	84,0
	0,0	0,0	3,0	7,0	24,0	50,0	80,0	91,0
	3,0	7,0	14,0	28,0	32,0	40,0	41,0	48,0
Mean	0,8	2	5	12	22	44	65	75
S.E.M.	0,8	2	3	6	4	3	9	9

$pD_{2:}$ 5.77 ± 0.27*

$E_{max:}$ 80 ± 11*

n = 4

* values are significantly ($p<0.05$) different from control

Table 10

Effect of 30 umol/l FLAVIN7® on the noradrenaline-induced increase of contractile tension of rabbit abdominal aorta

% increase of contraction after exposure to							
8	7.5	7	6.5	6	5.5	5	4.5
- log (mol/l) noradrenaline							
in the presence of FLAVIN7® (30 umol/l)							
1,0	4,0	8,0	18,0	27,0	43,0	63,0	66,0
2,0	7,0	13,0	27,0	43,0	68,0	93,0	95,0
1,0	7,0	12,0	26,0	38,0	62,0	71,0	73,0
1,0	6,0	12,0	30,0	40,0	62,0	87,0	90,0
0,0	0,0	3,0	6,0	21,0	42,0	63,0	68,0
0,0	0,0	0,0	9,0	19,0	38,0	67,0	77,0

Mean	0,8	4	8	19	31	53	74	78
S.E.M.	0,3	1	2	4	4	5	5	5

pD_2: 5.76± 0.09*

E_{max}: 87 ± 6*

n = 6

* values are significantly ($p<0.05$) different from control

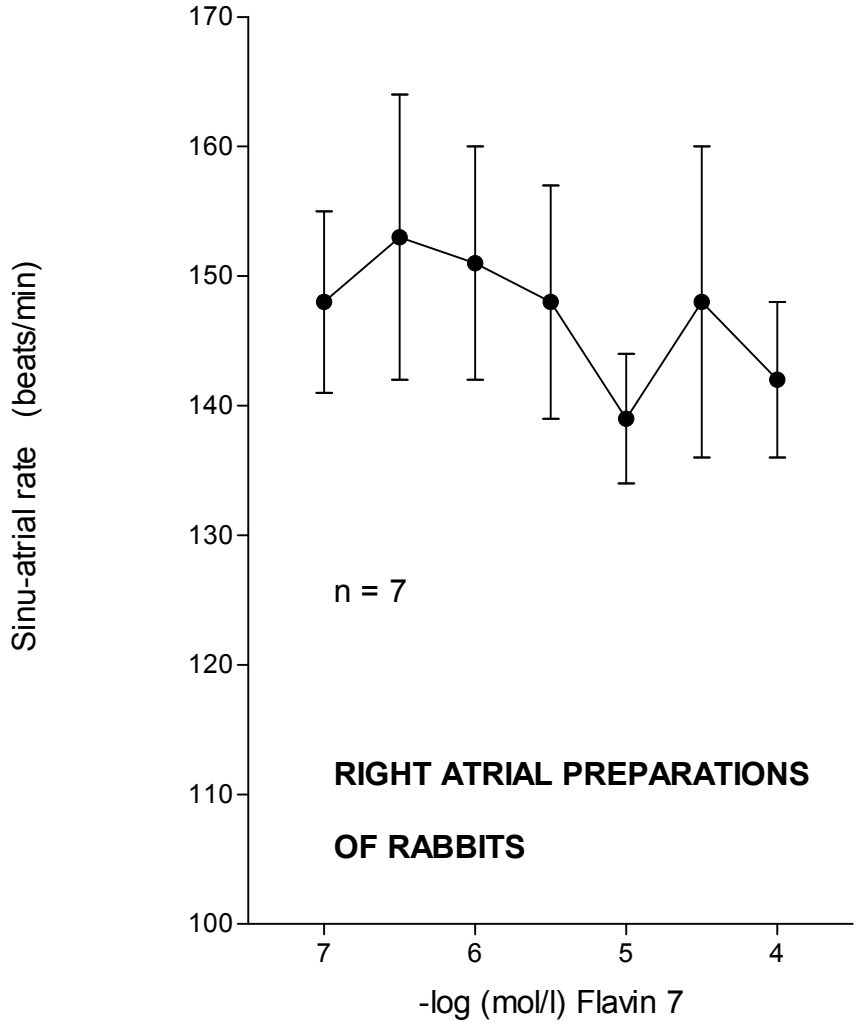

Fig. 12 Effect of various concentrations of FLAVIN7® on the sinu-atrial pacemaker activity of rabbits

Table 11

Effect of FLAVIN7® on the sinu-atrial pacemaker activity of rabbits

	sinu-atrial rate (beats/min) after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log(mol/l) FLAVIN7®						
	173	178	183	183	145	-	-
	135	-	138	133	133	-	-
	145	-	148	148	145	-	-
	165	165	163	160	153	153	150
	150	145	143	140	140	140	135
	153	163	175	163	140	178	155
	113	115	113	113	115	120	128
Mean	148	153	151	148	139	148	142
Std. Error	7	11	9	9	5	12	6

Control: 149 ± 7

n = 7

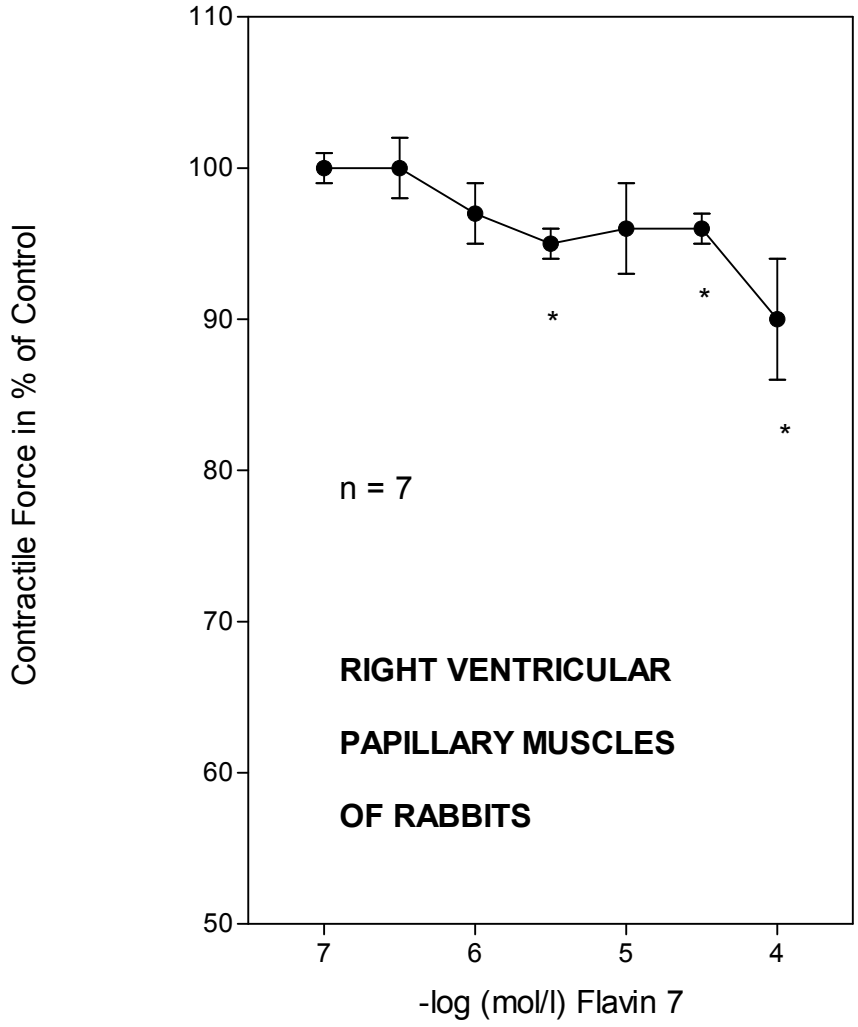

Fig. 13 Effect of various concentrations of FLAVIN7® on the contractile force of electrically driven right ventricular papillary muscles of rabbits (n = 7)

Table 12

Effect of FLAVIN7® on the contractile force in electrically driven right ventricular papillary muscles of guinea pigs (n = 7)

	Myocardial contractile force in % of pre-drug value after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) FLAVIN 7						
	106	103	104	100	98	92	83
	101	99	97	97	94	92	88
	96	100	96	96	95	91	86
	100	100	94	94	94	91	88
	104	106	102	102	111	111	113
	98	101	95	87	92	101	89
	97	91	92	90	90	93	85
Mean	100	100	97	95	96	96	90
S.E.M .	1	2	2	1	3	1	4
	n = 7						

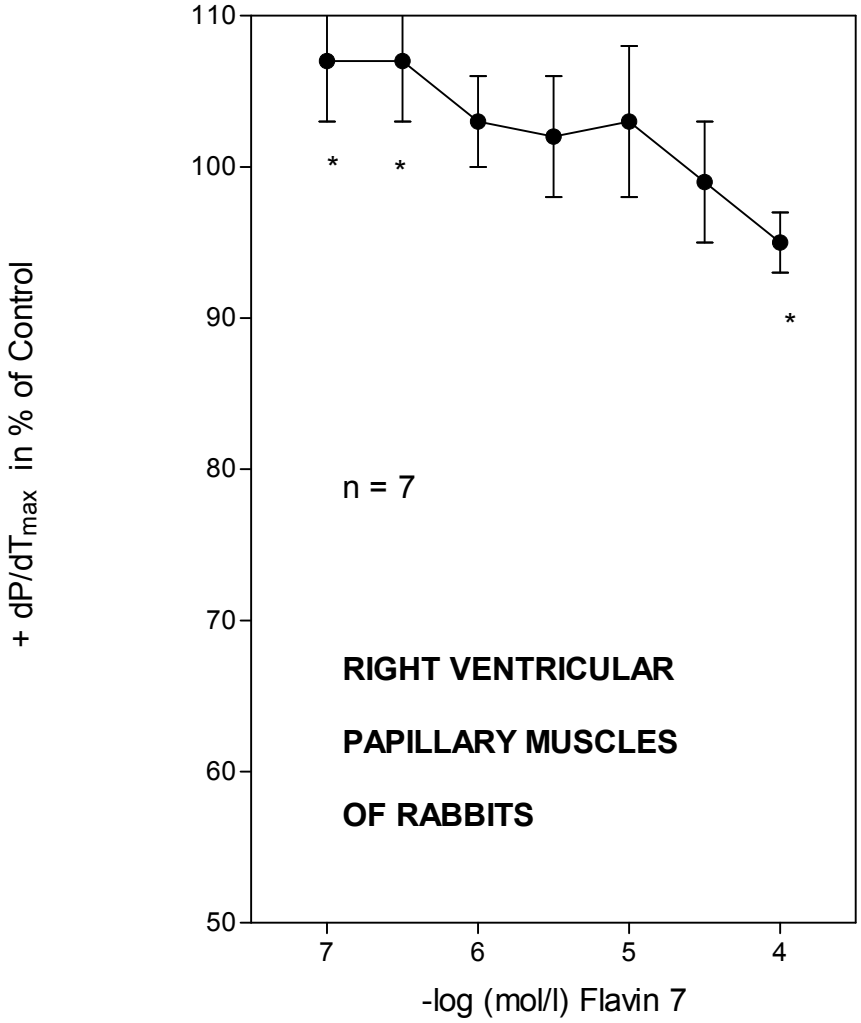

Fig. 14 Effect of various concentrations of FLAVIN7® on the maximum velocity of contraction (+dP/dTmax) of electrically driven right ventricular papillary muscles of rabbits (n = 7)

Table 13

Effect of FLAVIN7® on the maximum velocity of contraction (+dP/dTmax) in electrically driven right ventricular papillary muscles of guinea pigs (n = 7)

	+dP/dTmax in % of pre-drug value after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) FLAVIN 7						
	113	113	100	100	120	100	98
	116	110	100	98	94	90	90
	100	100	105	93	93	90	90
	120	120	120	120	120	120	100
	100	102	100	100	106	100	100
	110	110	106	110	100	100	100
	92	92	92	90	90	92	90
Mean	107	107	103	102	103	99	95
S.E.M .	4	4	3	4	5	4	2
	n = 7						

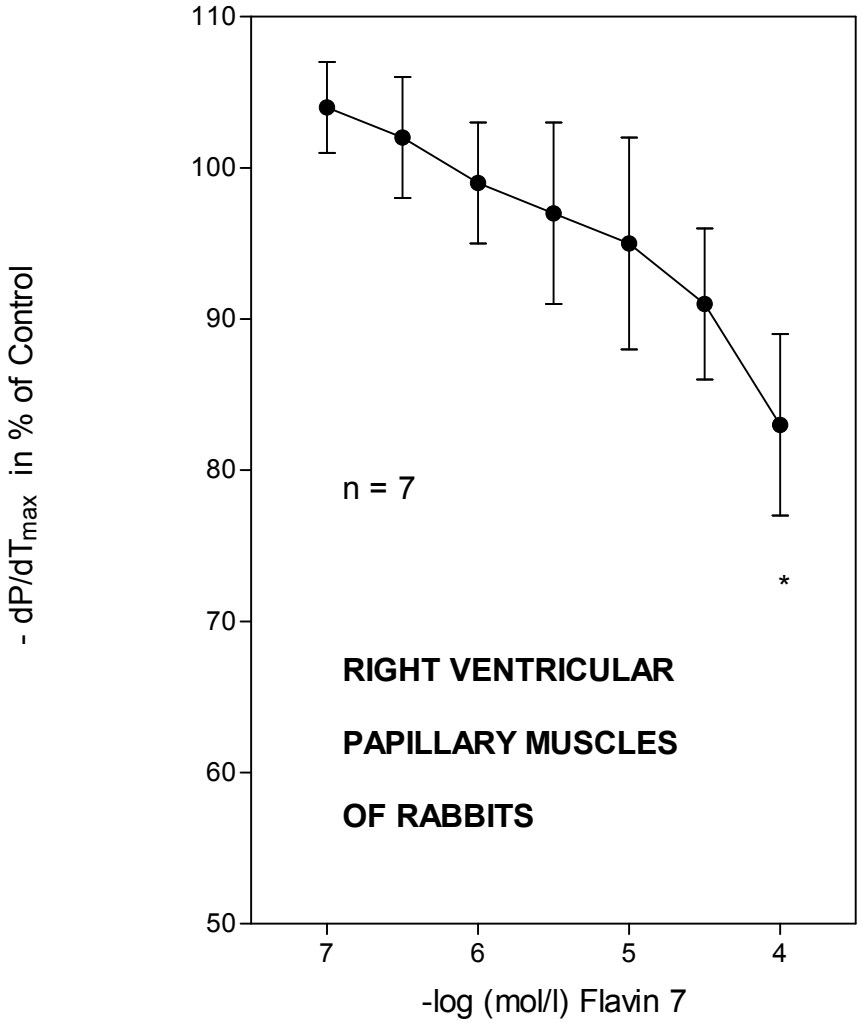

Fig. 15 Effect of various concentrations of FLAVIN7® on the maximum velocity of contraction (+dP/dTmax) of electrically driven right ventricular papillary muscles of rabbits (n = 7)

Table 14

Effect of FLAVIN7® on the maximum velocity of relaxation (-dP/dTmax) in electrically driven right ventricular papillary muscles of guinea pigs (n = 7)

	-dP/dTmax in % of pre-drug value after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) FLAVIN 7						
	117	97	92	92	83	83	67
	95	95	97	87	80	80	77
	98	98	91	88	83	83	71
	112	122	122	132	132	112	92
	105	105	98	100	105	105	112
	100	97	92	83	85	85	78
	100	100	100	100	95	87	82
Mean	104	102	99	97	95	91	83
S.E.M .	3	4	4	6	7	5	6

n = 7

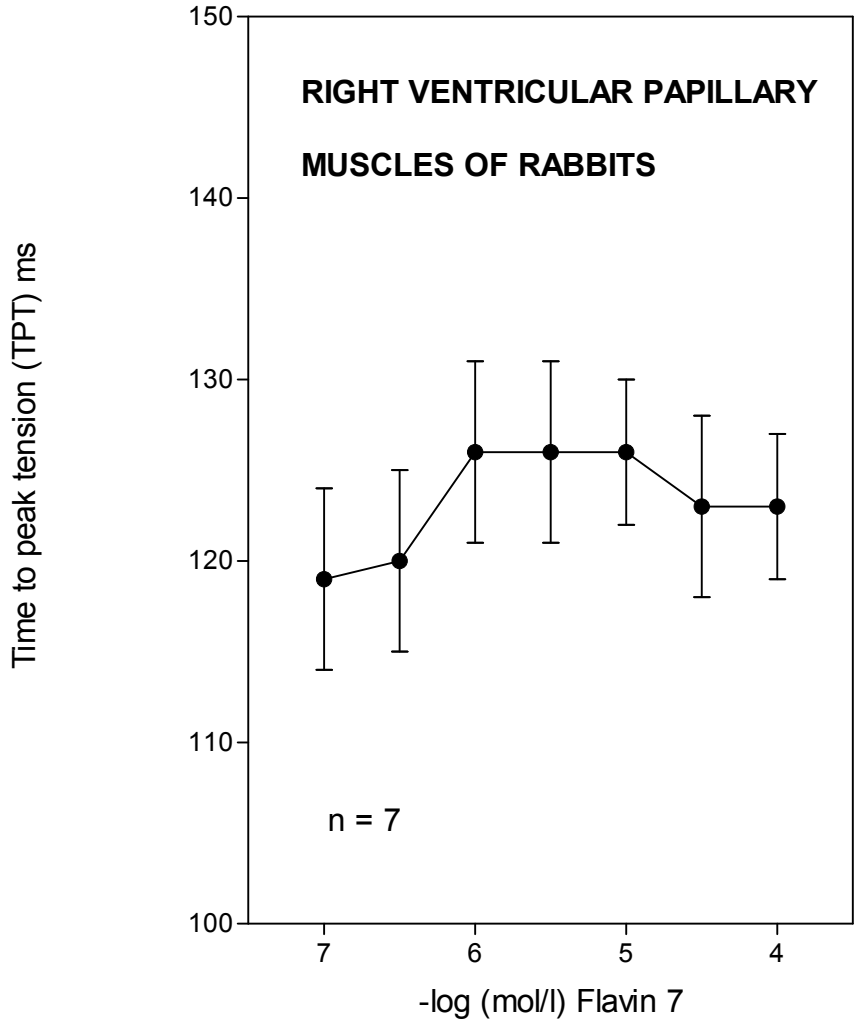

Fig. 16 Effect of various concentrations of FLAVIN7® on the time to peak tension (TPT) of electrically driven right ventricular papillary muscles of rabbits (n = 7)

Table 15

Effect of FLAVIN7® on the time to peak tension (TPT) in electrically driven right ventricular papillary muscles of guinea pigs (n = 7)

	TPT (ms) after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) FLAVIN 7						
	110	108	112	110	118	110	110
	125	125	140	140	130	130	130
	95	95	100	105	110	100	110
	130	130	135	140	140	130	125
	122	130	130	125	130	130	140
	125	120	130	125	120	130	125
	125	130	135	135	135	130	120
Mean	119	120	126	126	126	123	123
S.E.M .	5	5	5	5	4	5	4

Control: 121 ± 5

n = 7

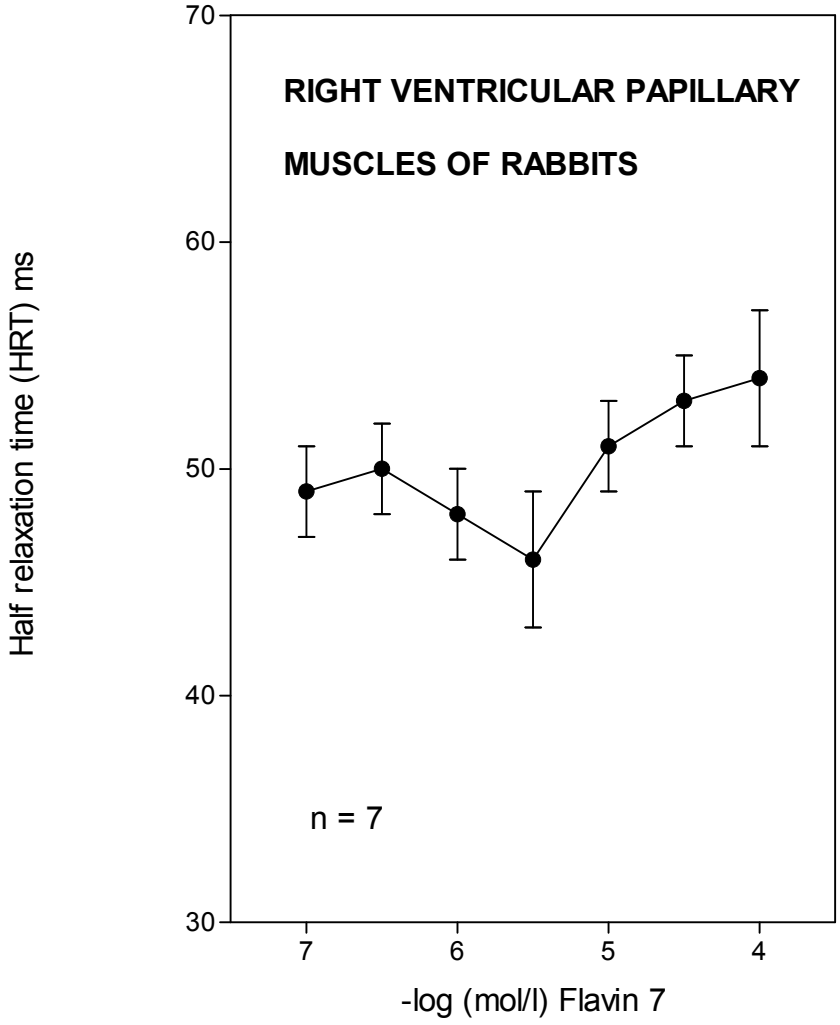

Fig. 17 Effect of various concentrations of FLAVIN7® on the half relaxation time (HRT) of electrically driven right ventricular papillary muscles of rabbits (n = 7)

Table 16

Effect of FLAVIN7® on the half relaxation time (HRT) in electrically driven right ventricular papillary muscles of guinea pigs (n = 7)

	HRT (ms) after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) FLAVIN 7						
	45	39	39	30	50	50	48
	58	58	55	53	59	59	59
	48	48	45	42	42	42	40
	50	48	45	40	45	55	60
	45	50	50	55	55	55	55
	55	55	50	50	55	60	55
	45	50	55	50	50	50	60
Mean	49	50	48	46	51	53	54
S.E.M .	2	2	2	3	2	2	3

Control: 50 ± 3

n = 7

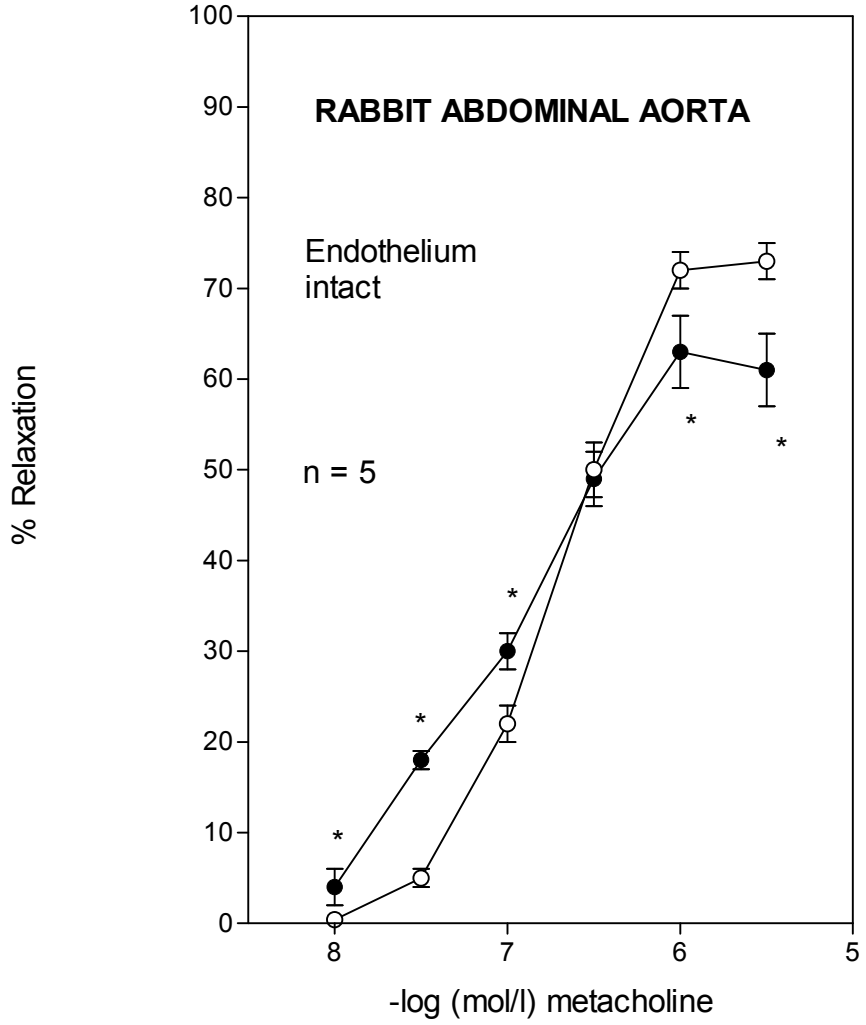

Fig. 18 Effect of 0.1 umol/l FLAVIN7® on the endothelium-dependent, metacholine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine

○ - FLAVIN7® (Control)

● + FLAVIN7®

n = 5

* significant difference ($p<0.05$) from control

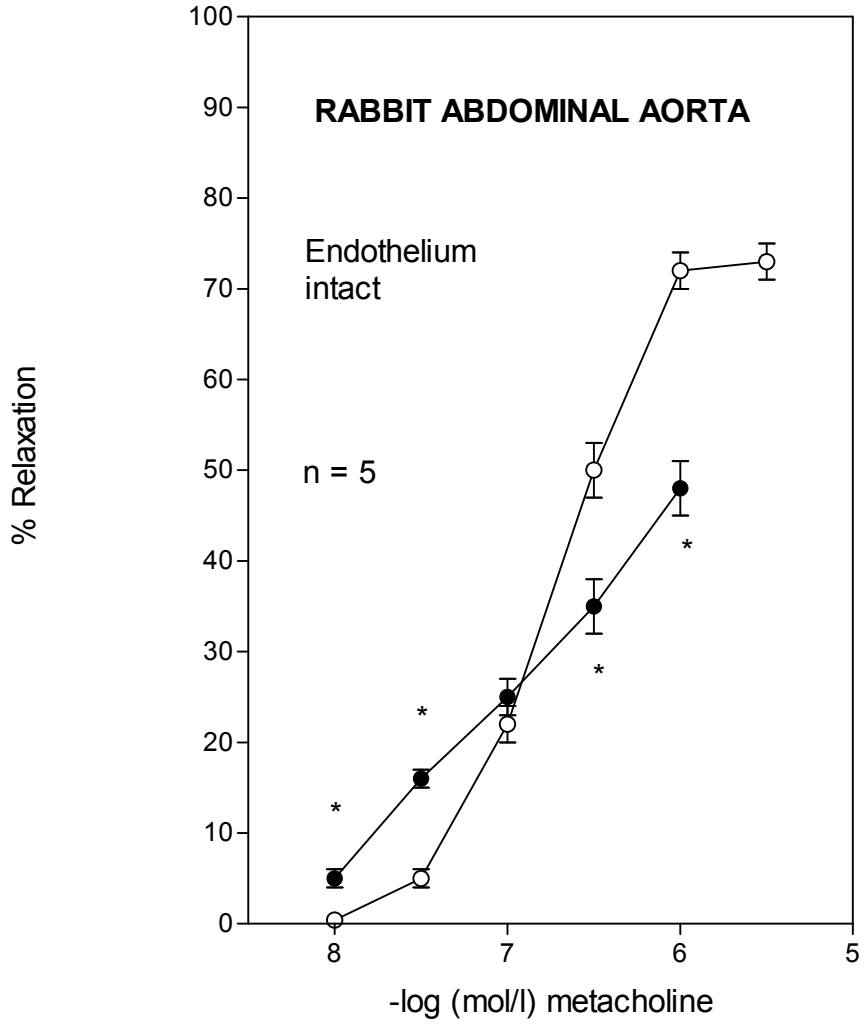

Fig. 19 Effect of 10 umol/l FLAVIN7® on the endothelium-dependent, metacholine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine

○ - FLAVIN7® (Control)

● + FLAVIN7®

n = 5

* significant difference ($p<0.05$) from control

Table 17

Effect of FLAVIN7® on the endothelium-dependent, metacholine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine (Control)

	relaxation in % of precontraction after exposure to					
	8	7.5	7	6.5	6	5.5
	- log (mol/l) metacholine					
	in the absence of FLAVIN7® (Control)					
	0,0	3,00	17,0	47,00	68,0	70,00
	2,0	8,00	27,0	53,00	72,0	73,00
	0,0	3,00	16,0	39,00	68,0	69,00
	0,0	7,00	24,0	55,00	76,0	76,00
	0,0	4,00	25,0	54,00	74,0	77,00
Mean	0,4	5	22	50	72	73
S.E.M.	0,4	1	2	3	2	2

n = 5

Table 18

Effect of 0.1 umol/l FLAVIN7® on the endothelium-dependent, metacholine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine

	relaxation in % of precontraction after exposure to					
	8	7.5	7	6.5	6	5.5
	- log (mol/l) metacholine					
	in the absence of FLAVIN7® (0.1 umol/l)					
	9,0	18,00	36,0	44,00	56,0	54,00
	5,0	22,00	30,0	51,00	65,0	65,00
	1,0	14,00	22,0	39,00	54,0	52,00
	1,0	19,00	32,0	55,00	71,0	68,00
	2,0	18,00	30,0	56,00	70,0	68,00
Mean	4	18	30	49	63	61
S.E.M.	2	1	2	3	4	3

n = 5

Table 19

Effect of 10 umol/l FLAVIN7® on the endothelium-dependent, metacholine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine

	relaxation in % of precontraction after exposure to				
	8	7.5	7	6.5	6
	- log (mol/l) metacholine				
	in the absence of FLAVIN7® (10 umol/l)				
	3,0	11,00	20,0	38,00	45,0
	7,0	18,00	25,0	39,00	54,0
	3,0	16,00	21,0	24,00	39,0
	7,0	18,00	30,0	37,00	53,0
	5,0	16,00	28,0	36,00	50,0
Mean	5	16	25	35	48
S.E.M.	0,9	1	2	3	3

n = 5

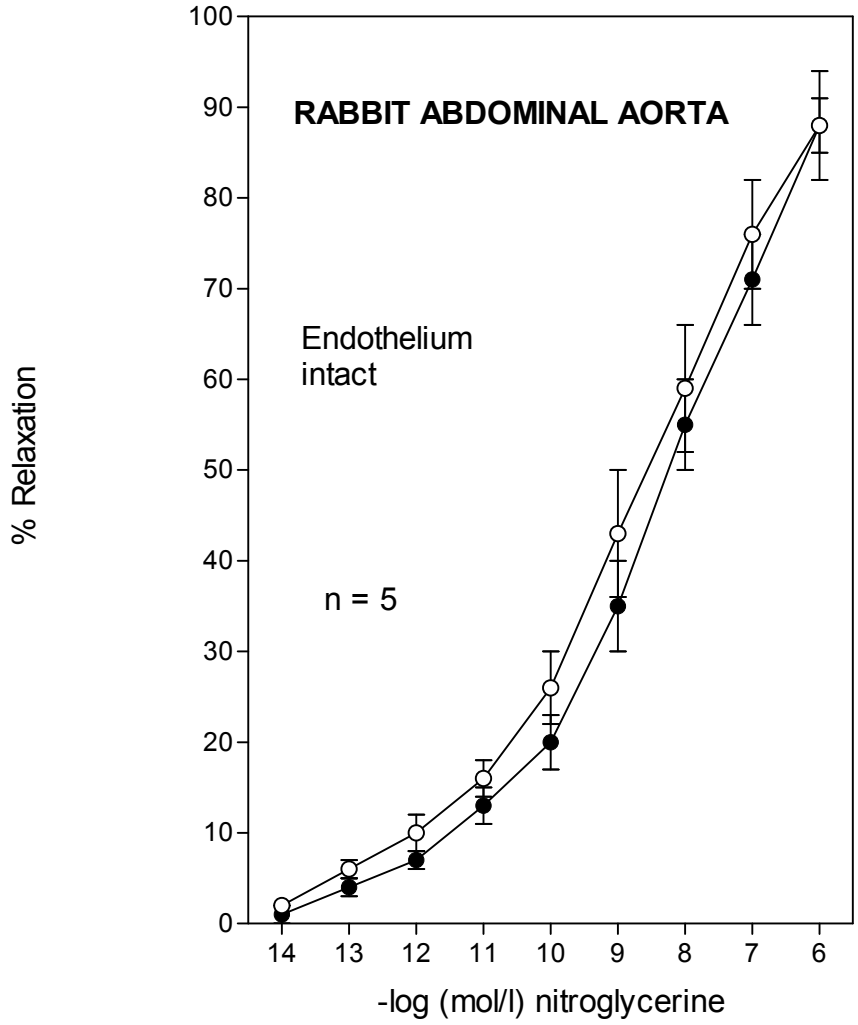

Fig. 20 Effect of 0.1 umol/l FLAVIN7® on the endothelium-independent, nitroglycerine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine

○ - FLAVIN7® (Control)

● + FLAVIN7®

n = 5

* significant difference ($p<0.05$) from control

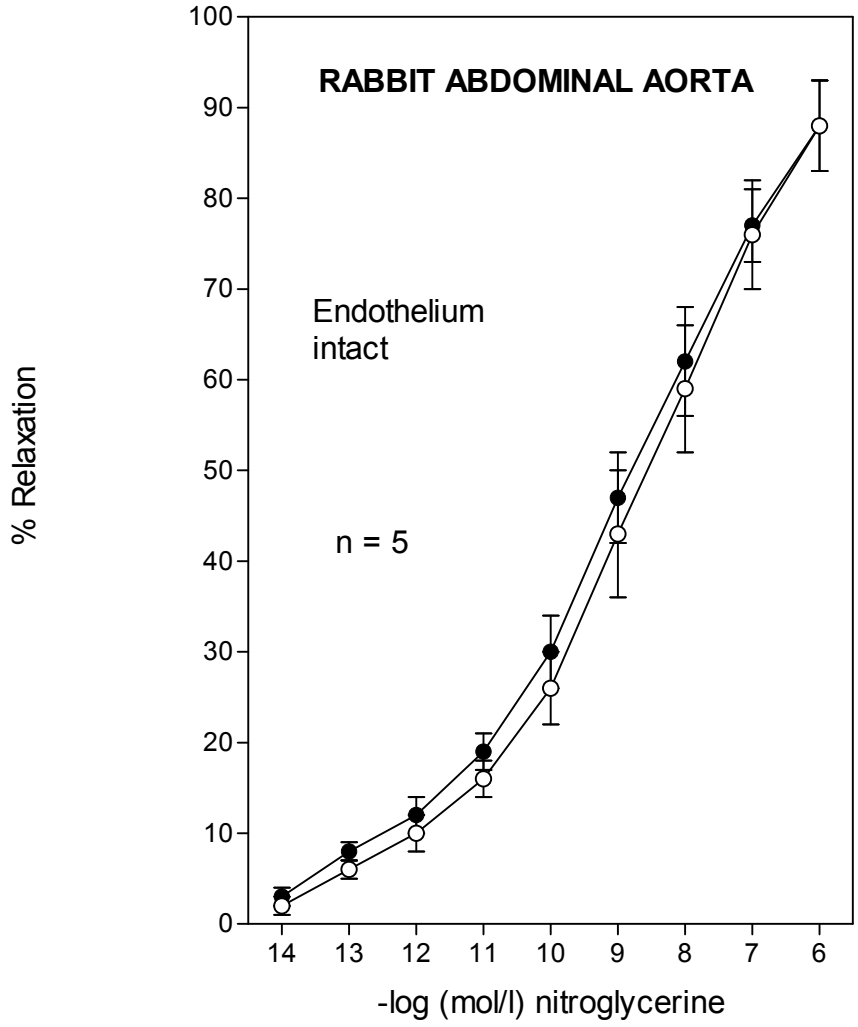

Fig. 21 Effect of 10 umol/l FLAVIN7® on the endothelium-independent, nitroglycerine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine

○ - FLAVIN7® (Control)

● + FLAVIN7®

n = 5

* significant difference ($p<0.05$) from control

Table 20:

Effect of FLAVIN7® on the endothelium-independent, nitroglycerine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine (Control)

	relaxation in % of precontraction after exposure to								
	14	13	12	11	10	9	8	7	6
	- log (mol/l) nitroglycerine								
	in the absence of FLAVIN7® (Control)								
	0,0	3,0	7,0	15,0	21,0	39,0	53,0	76,0	90,0
	2,0	4,0	6,0	10,0	18,0	30,0	45,0	58,0	70,0
	3,0	6,0	10,0	16,0	24,0	38,0	59,0	76,0	90,0
	2,0	11,0	17,0	23,0	42,0	68,0	83,0	96,0	100,0
	3,0	8,0	12,0	18,0	25,0	40,0	54,0	76,0	92,0
Mean	2	6	10	16	26	43	59	76	88
S.E.M.	0,5	1	2	2	4	6	6	6	5

n = 5

Table 21.

Effect of 0.1 umol/l FLAVIN7® on the endothelium-independent, nitroglycerine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine

	relaxation in % of precontraction after exposure to								
	14	13	12	11	10	9	8	7	6
	- log (mol/l) nitroglycerine								
	in the absence of FLAVIN7® (0.1 umol/l)								
	1,0	3,0	9,0	17,0	23,0	40,0	54,0	74,0	89,0
	0,0	2,0	3,0	6,0	12,0	21,0	46,0	56,0	79,0
	2,0	4,0	7,0	14,0	20,0	27,0	50,0	63,0	85,0
	0,0	3,0	7,0	10,0	17,0	43,0	73,0	85,0	97,0
	4,0	8,0	10,0	16,0	27,0	42,0	52,0	78,0	90,0
Mean	1	4	7	13	20	35	55	71	88
S.E.M.	0,7	1	1	2	3	4	5	5	3
	n = 5								

Table 22.

Effect of 10 umol/l FLAVIN7® on the endothelium-independent, nitroglycerine-induced relaxation in rabbit abdominal aorta precontracted with phenylephrine

	relaxation in % of precontraction after exposure to								
	14	13	12	11	10	9	8	7	6
	- log (mol/l) nitroglycerine								
	in the absence of FLAVIN7® (10 umol/l)								
	0,0	4,0	8,0	18,0	24,0	43,0	56,0	79,0	90,0
	3,0	6,0	9,0	19,0	22,0	38,0	50,0	65,0	72,0
	3,0	9,0	12,0	16,0	26,0	42,0	62,0	74,0	88,0
	3,0	11,0	21,0	26,0	46,0	67,0	84,0	89,0	100,0
	4,0	10,0	12,0	18,0	30,0	44,0	56,0	80,0	92,0
Mean	3	8	12	19	30	47	62	77	88
S.E.M.	0,7	1	2	2	4	5	6	4	5
	n = 5								

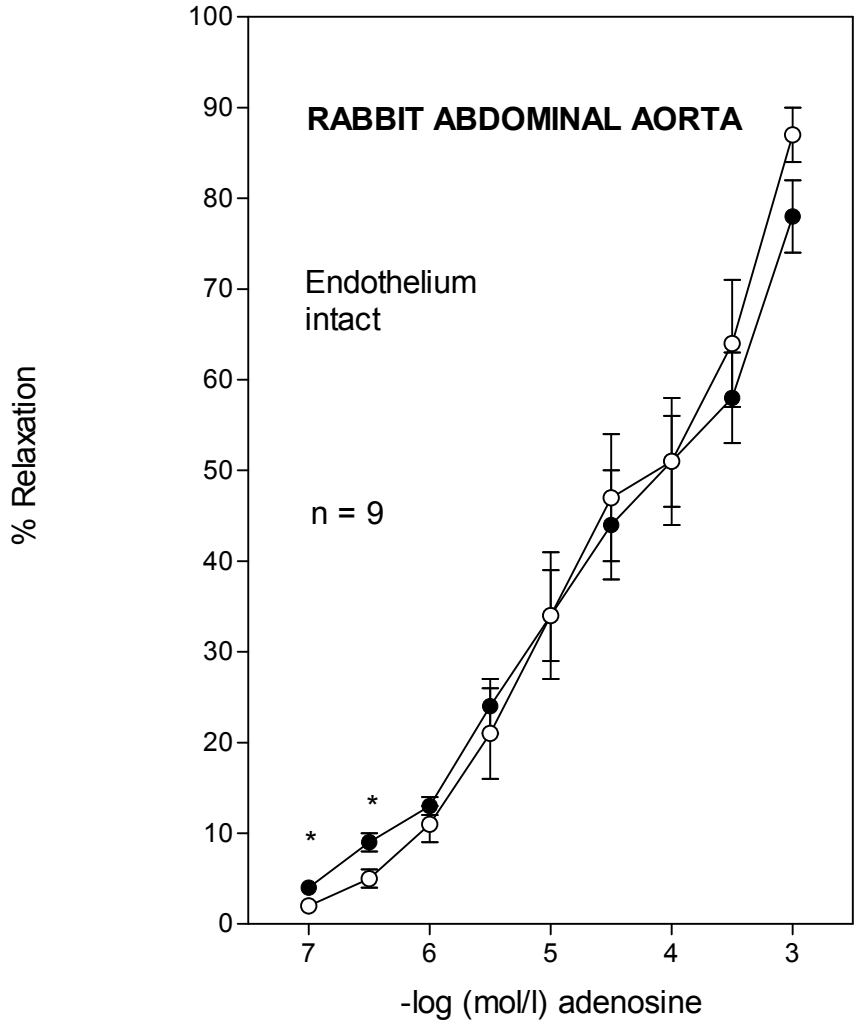

Fig. 22 Effect of 1 umol/l FLAVIN7® on the adenosine-induced relaxation in rabbit abdominal aorta

○ in the absence of FLAVIN7® (Control, n =9)

● in the presence of 1 umol/l FLAVIN7® (n = 9)

* Significant difference ($p<0.05$) from control

Table 23.

Effect of FLAVIN7® on the adenosine-induced relaxation in rabbit abdominal aorta (Control)

	% relaxation after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) adenosine						
	in the absence of FLAVIN7® (Control)						
	2,0	2,0	8,0	16,0	32,0	48,0	54,0
	4,0	5,0	7,0	10,0	15,0	24,0	34,0
	3,0	10,0	24,0	55,0	79,0	90,0	92,0
	1,0	2,0	5,0	7,0	14,0	24,0	31,0
	2,0	5,0	10,0	19,0	38,0	52,0	53,0
	3,0	7,0	17,0	33,0	50,0	61,0	62,0
	0,0	1,0	3,0	5,0	13,0	24,0	26,0
	1,0	3,0	7,0	13,0	20,0	37,0	47,0
	4,0	10,0	20,0	28,0	47,0	59,0	63,0
Mean	2	5	11	21	34	47	51
S.E.M.	0,5	1	2	5	7	7	7
	n =	**9**					

Table 24.

Effect of 1 umol/l FLAVIN7® on the adenosine-induced relaxation in rabbit abdominal aorta

	% relaxation after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) adenosine						
	in the presence of 1 umol/l FLAVIN7®						
	2,0	7,0	11,0	22,0	34,0	45,0	50,0
	3,0	7,0	10,0	16,0	23,0	31,0	39,0
	3,0	14,0	21,0	43,0	66,0	79,0	80,0
	6,0	10,0	13,0	18,0	24,0	26,0	32,0
	3,0	4,0	8,0	14,0	25,0	39,0	50,0
	2,0	7,0	17,0	30,0	43,0	53,0	57,0
	4,0	7,0	9,0	14,0	21,0	29,0	36,0
	7,0	14,0	16,0	25,0	32,0	41,0	50,0
	3,0	10,0	15,0	32,0	41,0	53,0	62,0
Mean	4	9	13	24	34	44	51
S.E.M.	0,6	1	1	3	5	5	5

n = 9

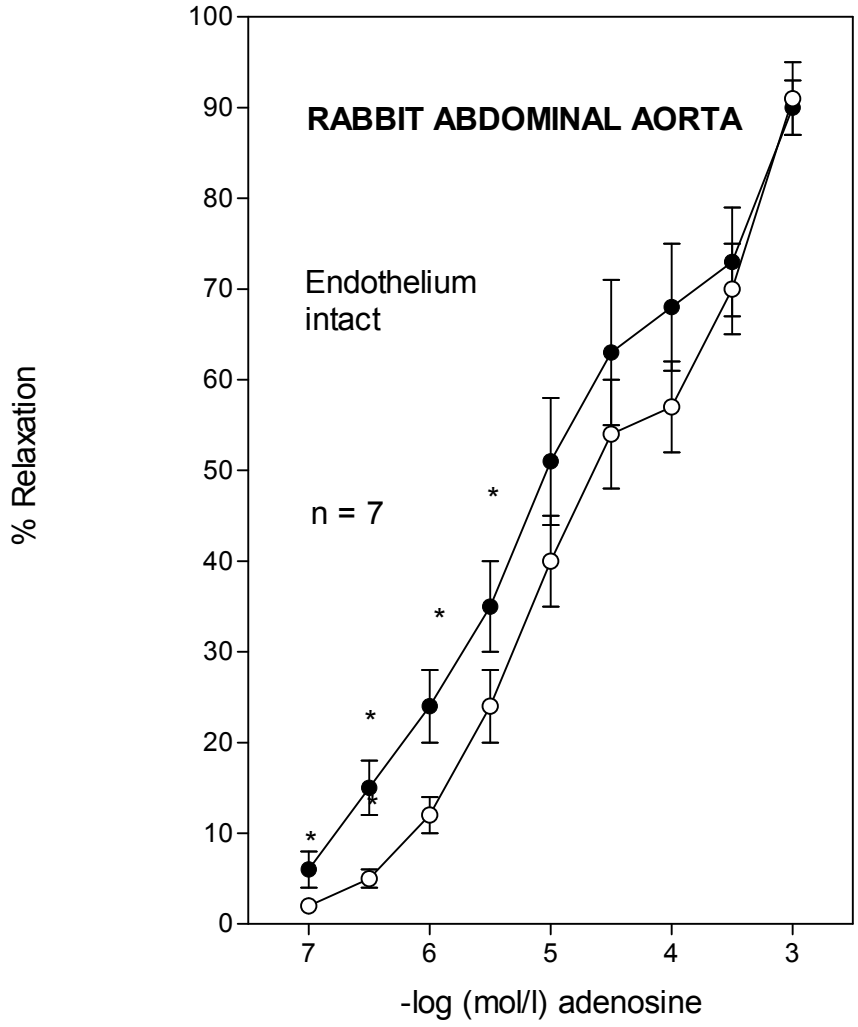

Fig. 23 Effect of 10 umol/l FLAVIN7® on the adenosine-induced relaxation in rabbit abdominal aorta

○ in the absence of FLAVIN7® (Control, n =7)

● in the presence of 10 umol/l FLAVIN7® (n = 7)

* Signifinant difference ($p<0.05$) from control

Table 25

Effect of FLAVIN7® on the adenosine-induced relaxation in rabbit abdominal aorta (Control)

	% relaxation after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) adenosine						
	in the absence of FLAVIN7® (Control)						
	2,0	2,0	8,0	16,0	32,0	48,0	54,0
	4,0	5,0	7,0	10,0	15,0	24,0	34,0
	2,0	6,0	16,0	32,0	46,0	64,0	72,0
	0,0	4,0	9,0	28,0	48,0	60,0	60,0
	2,0	9,0	15,0	33,0	50,0	67,0	67,0
	2,0	5,0	10,0	19,0	38,0	52,0	53,0
	3,0	7,0	17,0	33,0	50,0	61,0	62,0
Mean	2	5	12	24	40	54	57
S.E.M.	0,5	0,8	2	4	5	6	5
	n =	**7**					

Table 26.

Effect of 10 umol/l FLAVIN7® on the adenosine-induced relaxation in rabbit abdominal aorta

	% relaxation after exposure to						
	7	6.5	6	5.5	5	4.5	4
	- log (mol/l) adenosine						
	in the presence of 10 umol/l FLAVIN7®						
	1,0	7,0	14,0	27,0	39,0	47,0	54,0
	5,0	7,0	10,0	14,0	19,0	26,0	35,0
	7,0	15,0	22,0	35,0	53,0	67,0	70,0
	2,0	22,0	28,0	39,0	61,0	78,0	83,0
	9,0	26,0	32,0	35,0	50,0	65,0	71,0
	3,0	9,0	22,0	38,0	59,0	72,0	75,0
	13,0	22,0	39,0	57,0	74,0	83,0	87,0
Mean	**6**	**15**	**24**	**35**	**51**	**63**	**68**
S.E.M.	**2**	**3**	**4**	**5**	**7**	**7**	**7**
	n =	**7**					

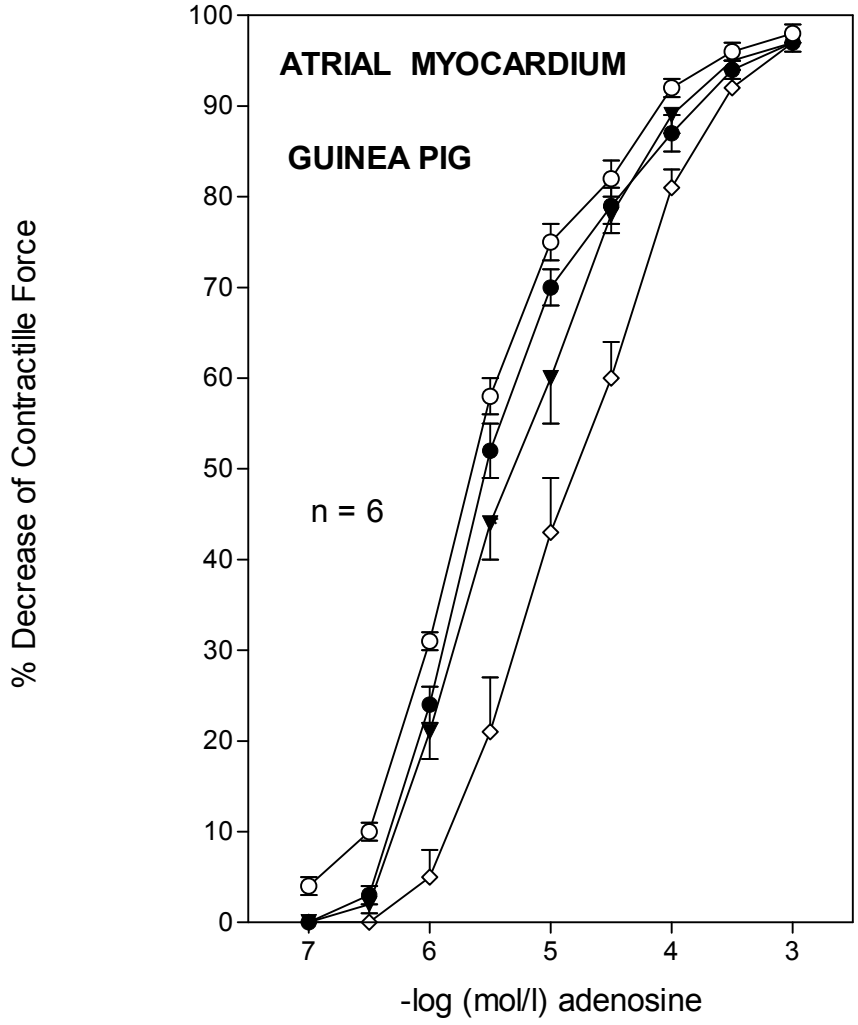

Fig. 24 Effect of various concentrations of FLAVIN7® on the concentration-response curves for adenosine in electrically driven left atrial preparations of guinea pigs.

○ in the absence of FLAVIN7® (n = 6)

● in the presence of 1 umol/l FLAVIN7® (n = 6)

▼ in the presence of 10 umol/l FLAVIN7® (n = 6)

◊ in the presence of 100umol/l FLAVIN7® (n = 6)

Values are expressed as % of the pre-adenosine values.

Table 27

Effect of FLAVIN7® on the adenosine-induced decrease of mechanical activity in electrically paced atrial myocardium of guinea pigs

	% decrease of myocardial contractile force after exposure to								
	7	6.5	6	5.5	5	4.5	4	3.5	3
	- log (mol/l) adenosine								
	in the absence of FLAVIN7® (Control)								
	3,0	6,0	26,0	49,0	66,0	77,0	89,0	94,0	97,0
	0,0	11,0	32,0	63,0	79,0	89,0	95,0	97,0	100,0
	5,0	14,0	32,0	59,0	77,0	82,0	91,0	95,0	100,0
	5,0	8,0	32,0	58,0	79,0	84,0	95,0	98,0	98,0
	4,0	8,0	30,0	57,0	75,0	80,0	92,0	96,0	96,0
	6,0	12,0	34,0	62,0	74,0	82,0	92,0	95,0	98,0
Mean	10	31	58	75	82	92	96	98	
S.E.M.	1	1	2	2	2	1	0,6	0,7	
	pD_2:	**5.71 ± 0.04**							
	n =	**6**							

Table 28

Effect of 1 umol/l FLAVIN7® on the adenosine-induced decrease of mechanical activity in electrically paced atrial myocardium of guinea pigs

	% decrease of myocardial contractile force after exposure to								
	7	6.5	6	5.5	5	4.5	4	3.5	3
	- log (mol/l) adenosine								
	in the presence of 1 umol/l FLAVIN7®								
	0,0	6,0	24,0	41,0	65,0	76,0	82,0	94,0	98,0
	0,0	5,0	30,0	60,0	77,0	85,0	95,0	95,0	98,0
	0,0	0,0	18,0	50,0	73,0	82,0	91,0	95,0	98,0
	0,0	5,0	29,0	52,0	66,0	71,0	81,0	90,0	96,0
	0,0	0,0	25,0	54,0	70,0	82,0	86,0	92,0	96,0
	0,0	0,0	18,0	56,0	68,0	78,0	88,0	95,0	98,0
Mean	0	3	24	52	70	79	87	94	97
S.E.M.	0	1	2	3	2	2	2	0,8	0,4

$pD_{2:}$ 5.47 ± 0.04*

n = 6

* Statistical difference ($p < 0.05$) from control values

Table 29.

Effect of 10 umol/l FLAVIN7® on the adenosine-induced decrease of mechanical activity in electrically paced atrial myocardium of guinea pigs

	% decrease of myocardial contractile force after exposure to								
	7	6.5	6	5.5	5	4.5	4	3.5	3
	- log (mol/l) adenosine								
	in the presence of 10 umol/l FLAVIN7®								
	0,0	5,0	15,0	33,0	58,0	70,0	82,0	94,0	98,0
	0,0	2,0	33,0	58,0	81,0	86,0	95,0	98,0	98,0
	0,0	0,0	12,0	40,0	47,0	81,0	91,0	95,0	98,0
	0,0	5,0	29,0	52,0	66,0	76,0	90,0	95,0	98,0
	0,0	0,0	18,0	38,0	52,0	74,0	88,0	96,0	98,0
	0,0	0,0	16,0	40,0	54,0	78,0	86,0	92,0	94,0
Mean	2	21	44	60	78	89	95	97	
S.E.M.	1	3	4	5	2	2	0,8	0,7	

pD_2: 5.39 ± 0.11*

n = 6

* Statistical difference ($p < 0.05$) from control values

Table 30.

Effect of 100 umol/l FLAVIN7® on the adenosine-induced decrease of mechanical activity in electrically paced atrial myocardium of guinea pigs

% decrease of myocardial contractile force after exposure to									
	7	6.5	6	5.5	5	4.5	4	3.5	3
	- log (mol/l) adenosine								
in the presence of 100 umol/l FLAVIN7®									
	0,0	0,0	12,0	24,0	47,0	65,0	82,0	94,0	98,0
	0,0	0,0	0,0	12,0	25,0	62,0	87,0	98,0	98,0
	0,0	0,0	0,0	12,0	35,0	47,0	76,0	88,0	98,0
	0,0	0,0	18,0	47,0	70,0	76,0	88,0	94,0	98,0
	0,0	0,0	2,0	14,0	42,0	52,0	78,0	88,0	96,0
	0,0	0,0	0,0	15,0	38,0	56,0	76,0	90,0	96,0
Mean	0,0	5	21	43	60	81	92	97	
S.E.M.	0,0	3	6	6	4	2	2	0,4	

pD_2: 4.83 ± 0.14*

n = 6

* Statistical difference ($p < 0.05$) from control values