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European Journal of Pharmacology 312 (1996) 203–207

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Involvement of monoamine oxidase and noradrenaline uptake in the positive chronotropic effects of apigenin in rat atria

Patricia S. Lorenzo [a,*], Modesto C. Rubio [b], Jorge H. Medina [c], Edda Adler-Graschinsky [a]

[a] *Instituto de Investigaciones Farmacológicas, CONICET, Junín 956, 5º piso, Buenos Aires 1113, Argentina*
[b] *Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina*
[c] *Instituto de Biología Celular, Facultad de Medicina, UBA, Buenos Aires, Argentina*

Received 1 February 1996; revised 19 June 1996; accepted 21 June 1996

Abstract

In rat isolated atria spontaneously beating and labelled with [^{3}H]noradrenaline, exposure to the flavonoid apigenin increased the atrial rate in a concentration-dependent manner (0.01–30 μM). This increase was accompanied by a reduction of 60% in the uptake of [^{3}H]noradrenaline as well as by a modification in the pattern of [^{3}H]noradrenaline and metabolites spontaneously released. Sixty minutes after exposure to 30 μM apigenin, the proportion of unmetabolized [^{3}H]noradrenaline increased from 11% to 45% of the total products collected in the organ bath whereas the tritiated *O*-methylated deaminated metabolites decreased from 33% to 14% of the total efflux. A small but significant decrease in the outflow of [^{3}H]3,4-dihydroxymandelic acid as well as a tendency to a decrease in the efflux of [^{3}H]3,4-dihydroxyphenylglycol was also observed. Furthermore, apigenin inhibited in a concentration-dependent manner the activity of monoamine oxidase in the rat atrial homogenates. The calculated IC50 (7.7 μM) was within the range that produced 50% of the maximal increase in atrial rate. It is concluded that apigenin possesses the property to increase the atrial rate, probably as a result of a reduction in noradrenaline uptake as well as in monoamine oxidase activity.

Keywords: Apigenin; Flavonoid; Monoamine oxidase; Noradrenaline uptake

1. Introduction

It has been reported that flavonoids, benzo-γ-pyrone derivatives that are widespread in the plant kingdom, possess a variety of pharmacological actions, such as anti-inflammatory, anti-allergic and anti-ulcer properties (Havsteen, 1983). Moreover, recent evidence indicates that the 5,7,4′-trihydroxyflavone apigenin, present in *Matricaria recutita*, exhibits anxiolytic as well as sedative properties in mice and binds to central benzodiazepine receptors with micromolar affinity (Viola et al., 1995).

Since binding sites to benzodiazepines have been observed on the rat heart (Taniguchi et al., 1982), and, in turn, benzodiazepines have been reported to exhibit cardiac actions, such as a decrease in the chronotropic response to noradrenaline in the rat atria (Elgoyhen and Adler-Graschinsky, 1989), the aim of the present work was to study the in vitro effects of apigenin on the chronotropic responses of atria isolated from rats.

2. Materials and methods

2.1. Animals and drugs

Wistar rats of 200–250 g body weight and of either sex were used. They were allowed free access to a standard laboratory diet and water.

Apigenin and tyramine hydrochloride were purchased from Sigma Chemical Co. (USA). (−)-Propranolol hydrochloride and (+)-propranolol hydrochloride were obtained from Imperial Chemical Industries, Pharmaceutical Division (UK). (±)-7-[^{3}H]Noradrenaline (specific activity: 10.5 Ci/mmol) and [ring-^{3}H]tyramine (specific activity: 33.4 Ci/mmol) were obtained from New England Nuclear Corp. (USA).

Apigenin was dissolved at a concentration of 1.92 mg/ml in an absolute ethanol : 3N KOH mixture (1 : 50) and diluted further with distilled water. Solutions of the remaining drugs were prepared daily in distilled water.

* Corresponding author. Tel.: +54-1-961-5949; fax: +54-1-963-8593; e-mail: postmaster@invfar.edu.ar

PII S0014-2999(96)00486-4

2.2. Tissue preparation

The animals were anesthetized with ethyl ether. The hearts were removed and the atria were dissected in Krebs solution of the following composition (mM): NaCl 118.0; KCl 4.7; $CaCl_2$ 2.6; $MgCl_2$ 1.2; NaH_2PO_4 1.0; $NaHCO_3$ 25.0; glucose 11.1; EDTA 0.004, and ascorbic acid 0.11. The temperature was kept at 37°C and the solution was bubbled with a 95%O_2-5%CO_2 mixture, so that its final pH was 7.4. The atria were set up in a 5-ml isolated organ bath and attached to a force-displacement transducer connected to a Grass Polygraph to record the spontaneous isometric contractions of the preparation. A period of equilibration of about 40 min was allowed to elapse until the basal resting rate varied by not more than 10 beats/min during a 10-min interval.

To study the effects of apigenin on the spontaneous chronotropic responses of the atria a cumulative concentration-response curve was made by means of stepwise increases in the concentration by a factor of three, at intervals of 5 min. In the experiments in which the β-adrenoceptors were blocked, propranolol was added 30 min before the addition of apigenin.

2.3. Radioactive experiments

The noradrenaline stores in the atria were labelled by incubation of the tissue for 30 min with 5 μCi/ml (0.5 μM) of (±)-7-[^{3}H]noradrenaline as described by Adler-Graschinsky et al. (1972).

After the incubation, the atria were washed eight times consecutively for 1 min and then nine times for 5 min before the collection of the samples was started. At the end of the experiments 4.5 ml of the bathing solution was used for chromatographic separation of noradrenaline and its metabolites on alumina and Dowex 50 × 4, according to the method described by Graefe et al. (1973). Five fractions were isolated: unmetabolized [^{3}H]noradrenaline, [^{3}H]3,4-dihydroxyphenylglycol, [^{3}H]3,4-dihydroxymandelic acid, [^{3}H]normetanephrine, and [^{3}H]*O*-methylated deaminated fraction which represents [^{3}H]4-hydroxy-3-methoxyphenylglycol plus [^{3}H]vanillic mandelic acid.

For studies of uptake and retention of [^{3}H]noradrenaline, the atria were placed in separate beakers containing 5 ml of Krebs solution, set in a metabolic incubator at 37°C and gassed with 95%O_2-5%CO_2. After preincubation of the atria for 15 min either in Krebs solution or in the presence of 30 μM apigenin, the incubation was continued for an additional 15 min with 0.1 μM (±)[^{3}H]noradrenaline. The tissue was then washed for 5 min in fresh Krebs solution, blotted, weighed, homogenized with 5 ml of 0.4 N perchloric acid containing EDTA (1 mg/ml) and Na_2SO_3 (1.25 mg/ml), and centrifuged for 10 min at 3000 × *g*. From the resulting supernatant, 0.5 ml aliquots were collected for determination of total counts and the remaining was used for determination of endogenous noradrenaline according to Laverty and Taylor (1968).

Monoamine oxidase activity was measured with 1 μM tyramine as substrate according to the method of McCaman et al. (1965).

2.4. Statistics

Student's *t*-test was used for two-sample comparisons and a P value smaller than 0.05 was regarded as significant.

3. Results

3.1. Effects of apigenin on the chronotropic responses of rat isolated atria

As shown in Fig. 1, exposure to apigenin produced a concentration-dependent increase in the atrial rate, which started at 1 μM apigenin and reached up to 70 beats/min when 30 μM apigenin was present. This positive chronotropic effect of apigenin is likely to be mediated through the activation of the postjunctional β-adrenoceptors, since it was significantly reduced by the β-adrenoceptor antagonist 0.3 μM (−)-propranolol. The inactive enantiomer 0.3 μM (+)-propranolol was without effect on the increases in atrial rate induced by apigenin (Fig. 2).

3.2. Effects of apigenin on the release of [^{3}H]noradrenaline from rat isolated atria

To analyse whether the increase in the atrial chronotropism caused by apigenin could result from the release of noradrenaline, the rat atria were labelled with [^{3}H]noradrenaline and the basal release of the transmitter was measured after the addition of the concentration of the

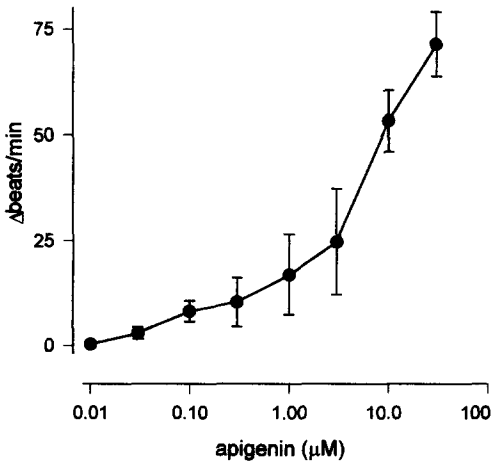

Fig. 1. Effects of apigenin on basal rate of rat isolated atria. Ordinate: increases in atrial rate in Δbeats/min. Abscissa: concentrations of apigenin in μM. Results shown are means ± S.E.M. (n = 5). The basal atrial rate was 263 ± 17 beats/min.

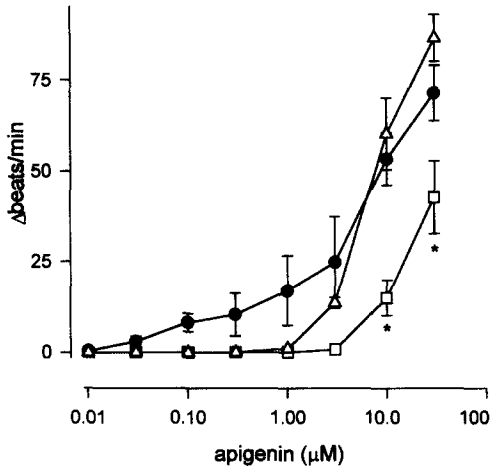

Fig. 2. Effects of propranolol on the positive chronotropic responses elicited by apigenin in rat isolated atria. Ordinate: increases in atrial rate in Δbeats/min. Abscissa: concentrations of apigenin in μM. Apigenin was added for periods of 5 min under control conditions (●) or after 30 min incubation with either 0.3 μM(−)-propranolol (□) or 0.3 mM (+)-propranolol (△). Basal atrial rates were 263 ± 17 beats/min in the controls, 269 ± 10 beats/min in the (−)-propranolol group and 224 ± 13 beats/min in the (+)-propranolol group. Results shown means ± S.E.M. of five experiments per group. The asterisks denote a significant difference ($P < 0.05$) compared to the corresponding control values.

flavonoid, 30 μM, that produced the maximal increase in the atrial rate.

As shown in Fig. 3 the basal efflux of radioactivity was not modified during the 60-min exposure to 30 μM apigenin. Nevertheless, apigenin modified the metabolic fate of the [^{3}H]noradrenaline released (Fig. 4). That is, during exposure to the flavonoid the efflux of total tritiated products collected as unmetabolized [^{3}H]noradrenaline increased from 11% under control conditions to 45% after the addition of 30 μM apigenin. This increase started 5 min after the apigenin exposure and remained to the end of the incubation, i.e., 60 min later. The increase in the proportion of unmetabolized [^{3}H]noradrenaline spontaneously released was accompanied by a reduction in the proportion of the [^{3}H]*O*-methylated deaminated fraction, which decreased from 33% under control conditions to 14% 60 min after the addition of apigenin. A small but significant decrease in the outflow of [^{3}H]3,4-dihydroxymandelic acid as well as a tendency to a decrease in the efflux of [^{3}H]3,4-dihydroxyphenylglycol was also observed.

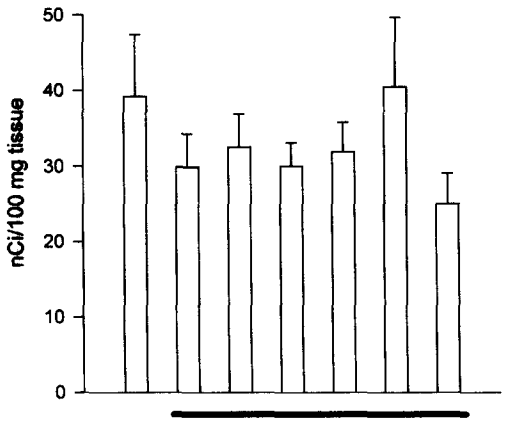

Fig. 3. Effects of apigenin on the spontaneous release of [^{3}H]noradrenaline from rat isolated atria. Ordinate: release of [^{3}H]noradrenaline in nCi/100 mg tissue. The tissues were exposed to 30 μM apigenin for 60 minutes. The duration of the exposure is indicated by the black rectangle at the bottom. The columns represent the spontaneous efflux in 5-min samples, collected at 10-min intervals. Results shown are means ± S.E.M. ($n = 4$).

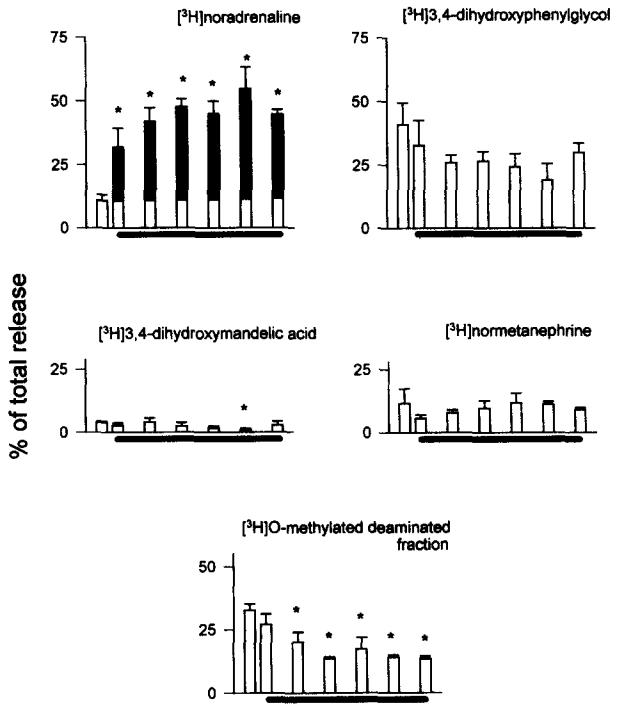

Fig. 4. Effects of apigenin on the spontaneous release of [^{3}H]noradrenaline and [^{3}H]metabolites from rat isolated atria. Ordinate: release of [^{3}H]noradrenaline and [^{3}H]metabolites expressed as a percentage of the total tritium efflux shown in Fig. 3. The tissues were exposed to 30 μM apigenin for 60 min. The duration of the exposure is indicated by the black rectangle at the bottom. The open columns represent the spontaneous efflux in 5-min samples collected at 10-min intervals, and the dark areas the increases above basal levels induced by apigenin incubation. Results shown are means ± S.E.M. from four experiments. The asterisks denote a significant difference ($P < 0.05$) compared to the basal value obtained immediately before the addition of 30 μM apigenin.

3.3. Apigenin on the activity of monoamine oxidase

To elucidate whether the effects of apigenin on the metabolism of [^{3}H]noradrenaline were due to a modification in the activity of monoamine oxidase, atrial homogenates were assayed for monoamine oxidase activity in the presence of different concentrations of apigenin. As shown in Fig. 5 the activity of monoamine oxidase was decreased in a concentration-dependent manner by 0.1–100 μM apigenin. The calculated IC_{50} (7.7 μM) was within

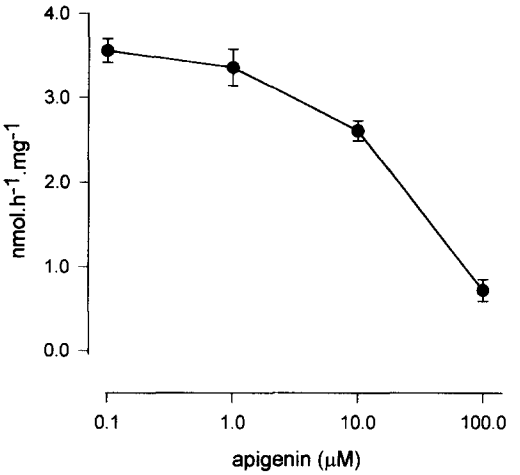

Fig. 5. Inhibition of in vitro monoamine oxidase activity by apigenin in rat atrial homogenates. Ordinate: enzyme activity expressed in nmol·h^{-1}·mg^{-1} (total activity). Abscissa: concentration of apigenin in μM. Monoamine oxidase activity was assayed in tissue homogenates with [^{3}H]tyramine as substrate. Apigenin was added simultaneously with the substrate. Results shown are means ± S.E.M. of four experiments.

the range that produced 50% of the maximal increase in the spontaneous atrial rate.

3.4. Effect of apigenin on the noradrenaline uptake in rat isolated atria

To study whether the interference with noradrenaline uptake could contribute, in addition to the inhibition of monoamine oxidase activity, to the increase in atrial rate produced by apigenin, rat atria were incubated in vitro with [^{3}H]noradrenaline and the retention of radioactivity was measured under control conditions and after the exposure to 30 μM apigenin.

As shown in Table 1, the uptake of [^{3}H]noradrenaline was reduced to 60% of control values after 15 min of preincubation with 30 μM apigenin. The endogenous content of noradrenaline was not modified by the apigenin exposure.

3.5. Effects of the repeated exposure to apigenin on the atrial chronotropism

To study the possibility that repeated exposure to apigenin could lead to tachyphylaxis to the chronotropic responses, as demostrated for the sympathomimetic amine tyramine in the rat atria (Lee et al., 1967), the tissue was repeatedly exposed to the concentration of the flavonoid (30 μM) that produced the maximal increase in atrial spontaneous frequency. The results obtained are depicted in Fig. 6 and showed that no tachyphylaxis was observed for the chronotropic effects of apigenin in the rat isolated atria.

Table 1
Effects of apigenin on the uptake of [^{3}H]noradrenaline in the rat isolated atria

Experimental group	n	Endogenous content [a]	Uptake [b]
Control	6	1.79 ± 0.22	4.84 ± 0.52
Apigenin 30 μM	6	1.78 ± 0.16	2.96 ± 0.50 [c]

Mean values ± S.E.M. are shown. [a] Endogenous content is expressed as μg noradrenaline/g wet tissue. [b] Uptake of [^{3}H]noradrenaline is expressed as ng [^{3}H]noradrenaline taken up/μg of endogenous noradrenaline. The atria were exposed to the flavonoid for 15 min and then incubated for 15 min with 0.1 μM (±)[^{3}H]noradrenaline. [c] $P < 0.05$.

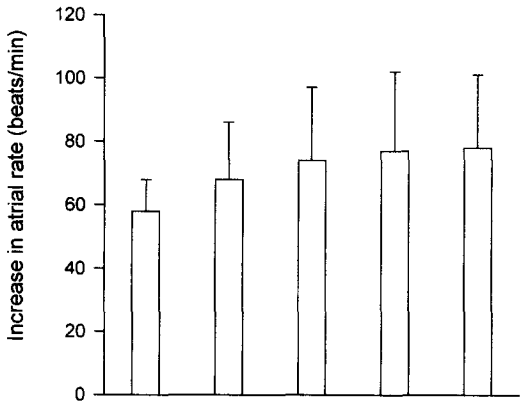

Fig. 6. Effect of repeated exposure to apigenin on the chronotropic responses of spontaneously beating rat atria. The atria were submitted to consecutive 5-min exposures to 30 μM apigenin (open bars) alternated with 10-min washes with Krebs solution. Basal atrial rate was 218 ± 32 beats/min. Results shown are means ± S.E.M. of three experiments.

4. Discussion

The results presented show that in rat isolated atria the basal chronotropic responses are increased by the flavonoid apigenin in a concentration-dependent manner.

The observation that in atria labelled with [^{3}H]noradrenaline apigenin caused a reduction in noradrenaline uptake as well as a modification in the pattern of release of neurotransmitter and metabolites, which was mainly accounted for by a pronounced increase in the proportion of unmetabolized [^{3}H]noradrenaline, could suggest that the positive chronotropic effect is in fact mediated through the activation by noradrenaline of the postjunctional β-adrenoceptors.

In further support of an indirect effect of apigenin is the observation that apigenin did not modify the binding of [^{3}H]dihydroalprenolol to rat atrial membranes, thus precluding the direct interaction of the flavonoid with β-adrenoceptors (Lorenzo and Medina, unpublished observations).

In addition, the possibility of a tyramine-like effect of apigenin is precluded by the observation that, differently to that observed with tyramine (Lee et al., 1967), the increases in atrial rate caused by apigenin were unmodified after repeated exposure to this flavonoid.

Dealing with the mechanism involved in the alteration caused by apigenin in the pattern of noradrenaline and

metabolites spontaneously released from the isolated atria, it appears that the inhibition of monoamine oxidase activity rather than the blockade of noradrenaline uptake is responsible for that effect. In this regard, during the spontaneous outflow of noradrenaline deamination takes place before noradrenaline reaches the synaptic gap (Langer, 1974) and consequently the inhibition of noradrenaline uptake does not modify the metabolism of noradrenaline as it was reported for the effects of cocaine on the spontaneous release of [^{3}H]noradrenaline from rat isolated atria (Pesce and Adler-Graschinsky, 1983).

Although in homogenated tissues deamination of noradrenaline is caused by monoamine oxidase-A as well as by monoamine oxidase-B, deamination is apparently restricted to monoamine oxidase-A in intact tissues (Osswald, 1987). In spite of the fact that the present results do not allow any characterization of the type of MAO that is inhibited by the flavonoid, it is tempting to speculate that monoamine oxidase-A, specially that located intraneuronally, could be the isoform involved in the apigenin effects. In this regard, it has been reported that in the guinea-pig atria the selective blockade of presynaptic monoamine oxidase by bretylium produces an increase in the proportion of unmetabolized [^{3}H]noradrenaline spontaneously released which is coincident with a rate-accelerating effect (Adler-Graschinsky et al., 1972).

In spite of the fact that, as far as we know, no inhibition of monoamine oxidase by flavonoids has been reported up to now, different flavonoid compounds have been proposed to inhibit oxide-reductase enzymes such as NADPH oxidase (Tauber et al., 1984). The latter effect could be due to the electrochemical property of flavonoids to transfer electrons (for review see Havsteen, 1983).

This possibility, which deserves further study, is of pharmacological relevance because several neuroactive compounds, such as clorgyline and moclobemide, used as antidepressive drugs are selective inhibitors of monoamine oxidase-A (for review see Baldessarini, 1984; Da Prada et al., 1990).

It is concluded that apigenin possesses, in addition to its capacity to bind to central benzodiazepine receptors and to exert anxiolytic effects (Viola et al., 1995), the property to increase the atrial rate, probably through the inhibition of monoamine oxidase activity as well as by the blockade of noradrenaline uptake. The reported effects could be of interest in the study of the potential therapeutic use of this group of substances.

Acknowledgements

The technical assistance of Ms Estela Torres and Ms Mónica Ferrari is gratefully acknowledged. This work was supported by Grant 3970 from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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