Comparison of Effects of Propranolol, Trimetazidine and Verapamil on the Contraction of Guinea-Pig Isolated Vas Deferens

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Summary

1. The effects of propranolol, verapamil and trimetazidine were compared on calcium-induced and potassium-induced contractions of guinea-pig isolated vas deferens in a sucrose medium. The preparations were pretreated with tetrasodium edetate before eliciting calcium-induced contractions.

2. Propranolol (0.1 mM) and verapamil (5 pM) depressed potassium-induced contractions, but did not depress calcium-induced contractions until the concentration of the antagonist was increased at least ten-fold.

3. Trimetazidine (3 pM) depressed calcium-induced contractions, but increased potassium-induced contractions.

Introduction

The β-adrenoceptor antagonist propranolol (Shanks, 1966)\(^1\), the calcium antagonist verapamil (Kohlhardt et al., 1972)\(^2\) and the anti-anginal drug trimetazidine (Schmitt 1964)\(^3\) have a number of clinical uses in common. We set out, therefore to compare their effects on smooth muscle contractions, using a preparation of the guinea-pig isolated vas deferens we have described previously (Sugimoto, Hanasaki & Shintani, 1980)\(^4\). The contractions were calcium-induced (Sugimoto, Furumichi & Murakami, 1978 a)\(^5\) and potassium-induced (Sugimoto & Nagata, 1973\(^6\); Sugimoto & Furumichi, 1975)\(^7\). The former were induced by the addition of CaCl\(_2\) to the preparation, pretreated with tetrasodium edetate and then transferred to edetate-free sucrose medium, and was assumed to be caused by the influx of calcium ions from the medium. The latter were induced by the addition of KCl to the preparation kept in a sucrose medium without edetate-prepreatment, and was assumed to be caused by the release of calcium ions within the tissue. If the assumptions are correct, drugs with selective effects
on the influx or release of calcium ions in the smooth muscle should have different effects on the two types of contractions.

**Methods and Results**

Male adult guinea-pigs weighing 400—450g were killed by a blow on the head. Their vasa deferentia were dissected out and suspended in an organ bath containing Locke’s solution saturated with pure oxygen at 30°C; the pH was 7.3 to 7.5. Contractions were recorded on a smoked drum with a lever exerting a tension of about 0.1g. The constituents (mM) of the Locke’s solution used were NaCl 154, KCl 5.5, CaCl₂ 2.2, NaHCO₃ 8.3, and glucose 5.5 in distilled water. The constituents of the isotonic sucrose medium were sucrose 325.7, NaHCO₃ 8.3, and glucose 5.5 in distilled water; the pH was 7.3 to 7.5. The drugs used were tetrasodium edetate (Tokyo Chemicals), propranolol hydrochloride (Sawatal®, Sawai Pharmaceutical), trimetazidine hydrochloride (Vastarel-F®, Sumitomo Pharmaceutical) and verapamil hydrochloride (Eizai).

Before inducing a contraction with CaCl₂, the preparation was pretreated with tetrasodium edetate in a concentration of 0.01 (w/v) in the sucrose medium for 20 min and then returned to the edetate-free sucrose medium; CaCl₂ was added 5 min later. The CaCl₂ was added at 20 s intervals in cumulatively increasing concentrations: 0.55, 0.55, 1.1, 2.2, 4.4, 8.8, and 17.6 mM.

The KCl-induced contraction was tested without edetate-pretreatment, 20 min after changing to the sucrose medium. The KCl was added at 20 s intervals in cumulatively increasing concentrations: 1.4, 1.4, 2.8, 5.6, 11.2, 22.4, and 44.8 mM.

Drugs tested were added 5 min before each CaCl₂- or KCl-induced contractions were elicited. Contractions were expressed as a percentage of the maximal contraction induced by noradrenaline in the same preparation kept in Locke’s solution. Propranolol (0.01—0.1 mM) depressed KCl-induced contractions in a concentration-dependent manner and completely abolished them in a concentration of 0.1 mM (Fig. 1b). However, propranolol in concentrations up to 0.1 mM had no effect on CaCl₂-induced contractions, although high concentrations (1.0 mM or more) did depress the CaCl₂-induced contraction (Fig. 1a).

Verapamil had similar effects to those of propranolol: it depressed KCl-induced contractions in a concentration-dependent manner over the range 0.05 to 0.5 μM, and completely abolished the contractions in a concentration of 2.0 μM (Fig. 1f). However, a high concentration of 50 μM was required to depress CaCl₂-induced contractions (Fig. 1e).

On the other hand, trimetazidine (0.003 mM) depressed CaCl₂-induced contractions whereas it increased KCl-induced contractions (Fig. 1e, d).
Fig. 1 Effects of propranolol, trimetazidine, and verapamil on CaCl₂-induced and KCl-induced contractions of guinea-pig isolated vas deferens. The CaCl₂-induced contractions were elicited in preparations pretreated with edetate and then transferred to edetate-free sucrose medium. The KCl-induced contractions were elicited in preparations kept in the sucrose medium without edetate-pretreatment. Propranolol, trimetazidine and verapamil were added to each medium 5 min before eliciting contractions. The concentrations shown against each curve are in mM. Each contraction was expressed as a percentage of the noradrenaline-induced maximal contraction in the same preparation. Each point is the mean of the contractions produced 20 s after addition of CaCl₂ or KCl in cumulative concentrations. The vertical lines are the standard errors of the means from values in six preparations. (In some cases the standard error is smaller than the symbol dimension). •—• control.

**Discussion**

Generally speaking, calcium ions utilized in smooth muscle contraction are supplied either from the medium or from within the tissue. When the contraction occurs in a calcium-free medium, the calcium ion can only be supplied from the tissue. This is the case with potassium-induced contractions of guinea-pig isolated vas deferens in a sucrose medium (Sugimoto et al., 1973, 1975). On the other hand, when the preparation is depleted of membrane calcium by pretreatment with tetrasodium edetate and then transferred to edetate-free sucrose medium, the addition of CaCl₂ induces a contraction (Sugimoto et al., 1978a) which is presumably caused by the influx of calcium ions from the medium. Differences between the potassium- and calcium-induced contractions were discussed by Sugimoto et al. (1980), who pointed out that sodium ions markedly depressed potassium-induced contractions but only slightly reduced calcium-induced contractions.

In sufficiently high concentrations, propranolol, trimetazidine, and verapamil had a suppressive effect on both the calcium- and potassium-induced contraction. This suggests that each drug may inhibit not only the influx of calcium ions from the medium but also the release of calcium ions from the tissue. However, with each of these drugs, there were concentrations having selective actions. Thus, potassium-induced contractions were selectively depressed by propranolol (0.1 mM) and also by verapamil (0.002 mM) in concentrations that did not affect calcium-induced contractions: a ten-fold higher concentration of each drug was needed to depress calcium-induced contractions. These results suggest that both drugs inhibit the release of membrane calcium ion more markedly than the influx of calcium ion. Trimetazidine had a different pattern of action: a low concentration (0.003 mM) depressed calcium-induced contractions but increased potassium-induced contractions. Therefore, it can be assumed that trimetazidine inhibits both the influx and the efflux of calcium ion.

It is generally accepted that verapamil inhibits calcium influx into cardiac muscle (Kohlhardt et al., 1972). However, calcium influx in our preparation of the vas deferens depends on the reduction of membrane calcium (Sugimoto et al., 1978a). When the release of membrane calcium ion is inhibited by verapamil, a decrease in calcium influx would indirectly result. It is significant that the effect of sodium ion on the release of membrane calcium ion (Sugimoto et al., 1975, 1978b) resembles those of propranolol and verapamil on the smooth muscle contraction.

**References**

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