EFFECTS OF CALCIUM-ANTAGONISTIC CORONARY VASODILATORS ON MYOCARDIAL CONTRACTILITY AND MEMBRANE POTENTIALS

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Abstract To clarify the relation between the negative inotropic effects of "calcium-antagonistic" vasodilators and their calcium-antagonistic effects, the effects of nifedipine, verapamil and diltiazem on isolated electrically-driven left atrial preparations of the guinea pig were studied. The ion-specificity of the antagonistic effects was also studied. In normal Tyrode's solution, all three vasodilators produced a shift to the right of the dose-response curve for calcium, the pA2 values being 5.90 for nifedipine, 4.88 for verapamil and 4.07 for diltiazem. The maximum rate of rise of action potentials recorded as a measure of the sodium permeability of the membrane was found to be reduced by verapamil and diltiazem, while this rate was unaffected by nifedipine. All three vasodilators suppressed the contractile activities induced in potassium-depolarized atria by isoproterenol and the dose-response curves for calcium were shifted to the right, the pA2 values being 8.24 for nifedipine, 6.67 for verapamil and 6.57 for diltiazem. In another set of experiments, calcium-dependent action potentials were evoked in the potassium-depolarized atria either by isoproterenol or aminophylline. These action potentials were suppressed by the above three vasodilators at dosage levels comparable to those producing suppression of the isoproterenol-induced contractile response of the depolarized atria.

Since the pioneer work of Fleckenstein and his collaborators (1), which showed that three recently-introduced vasodilators, namely, prenylamine, verapamil and D-600, could produce a suppression of the contraction of the isolated papillary muscle preparation of the guinea pig without producing any remarkable change in the height and shape of the action potential and that the effects could be counteracted either by increasing the calcium concentration of the bathing medium or by administration of isoproterenol, special attention was directed to a group of vasodilators with similar modes of action (2-15) and the collective term "calcium-antagonists" was given to these substances.

The present study was undertaken to correlate the negative inotropic action of three representative calcium-antagonistic vasodilators, namely nifedipine, verapamil and diltiazem, to their Ca-antagonistic action, and to determine whether or not the antagonistic effects are specifically directed to calcium.

MATERIALS AND METHODS

Male albino guinea pigs weighing 300–450 g were sacrificed by a blow on the head. The hearts were removed quickly and placed in Tyrode's solution of the following composition (in mM): KCl, 2.7; NaCl, 137; CaCl2, 1.9; MgCl2, 1.0; NaHCO3, 11.9; NaH2PO4, 0.4;
glucose, 5.5, aerated with 95% oxygen + 5% carbon dioxide and the left atrium was dissected out. The following two types of experiments were performed.

**Contractile force measurement**

*Experiments in normal Tyrode’s solution:* The left atrium was suspended in an organ bath (20 ml) containing Tyrode’s solution aerated with 95% O₂ + 5% CO₂ and kept at a temperature of 30±1°C. The contractile tension of the preparation was recorded on an ink-writing oscillograph with a strain-gauge transducer and a carrier amplifier. A field stimulation technique was employed to ensure synchronous excitation of the whole muscle fiber. Two silver plate electrodes (5 x 10 mm) separated by 6 mm were placed parallel to the muscle and the preparation was stimulated at the frequency of 1 Hz with voltage approx. 30% above the threshold (duration = 1 msec). The resting tension was kept at 0.25 g throughout the course of the experiment.

*Experiments in high-potassium medium:* The effects of calcium antagonistic vasodilators on the contractile tension of the depolarized atria were studied in the following way: After one hour equilibration period in normal Tyrode’s solution, the bathing solution was switched to a high potassium (22.0 mM) Tyrode’s solution and frequency of stimulation was reduced to 0.4 Hz (duration 3-4 msec). To maintain the isoosmotic condition, sodium concentration of the bathing medium was reduced to 118.0 mM. Under this condition the preparation was depolarized and the contraction disappeared in less than 2 or 3 min. After further equilibration of 45 min, isoproterenol (0.2 µM) or aminophylline (1.1 mM) was added to the bathing solution. Contractile activities resumed in association with calcium-dependent action potentials.

**Measurements of membrane potential**

*Experiments in normal Tyrode’s solution:* The left atrial muscle strips were pinned with the endocardial surface uppermost to a paraffin block in an organ bath (10 ml). Tyrode’s solution, aerated with 95% O₂ + 5% CO₂, flowed through the organ bath at a rate of 4 ml/min. The temperature of the organ bath was maintained at 30±1°C (10). The muscle was stimulated with square wave pulses of an electronic stimulator (1 Hz; duration = 1 msec).

Evoked action potentials were recorded with a glass capillary micro-electrode filled with 3 M KCl, the electrical resistance of which was 10–40 MΩ, and were displayed on a cathode ray oscilloscope. To record the maximum rate of rise (Vmax), the output of the DC amplifier of the oscilloscope was fed to a R-C circuit, the time constant of which was 20 µsec, and then applied to the second channel of the oscilloscope.

*Experiments in high-potassium (22.0 mM) Tyrode’s solution:* Calcium-dependent action potentials were evoked in high-potassium Tyrode’s solution adding either isoproterenol or aminophylline and were displayed on an oscilloscope as described above.

Drugs used were l-isoproterenol hydrochloride (Proterenol, Nikken Kagaku), aminophylline (Neophylline, Eisai), nifedipine (Bayer), verapamil (Vasolan, Eisai), diltiazem (Herbesser, Tanabe) and tetrodotoxin. The last compound was kindly provided by Sankyo Pharmaceutical Co. The concentrated solution (0.1 ml) of these compounds was added to the organ bath.
RESULTS

Effects of calcium antagonistic vasodilators on the atrial contraction in normal Tyrode’s solution

The dose-response relation of the contractile tension development of the atrial muscle preparation for calcium (0.6–9.0 mM) was first obtained in normal Tyrode’s solution, then, the dose-response curve for calcium was again obtained after addition of vasodilators. Fig. 1 represents dose-response curves for calcium as affected by a dose of $1 \times 10^{-6}$ g/ml of nifedipine. As is evident from this figure, there was a shift to the right of the dose-response curves under the influence of this compound. This was also the case with verapamil and diltiazem. Although the shift was not strictly parallel, pA₂ values were calculated using the following equation.

$$pA_2 = -\log \frac{[C]}{[B]}$$

where A is dose of Ca⁺⁺ at which the contraction was 50% of maximum contraction, B is dose of Ca⁺⁺ at which the contraction was 50% of maximum contraction in the presence of antagonists, and C is dose of antagonists. The pA₂ values of nifedipine, verapamil and diltiazem were 5.90, 4.88 and 4.07, respectively, indicating that the Ca-antagonistic potency in normal Tyrode’s solution was in the following order: nifedipine > verapamil > diltiazem (Table 1).

Effects on the contractility of depolarized atrial muscle

When atrial muscles were exposed to Tyrode’s solution containing 22 mM K⁺, they

### Table 1. pA₂ values of calcium antagonistic effects of nifedipine, verapamil and diltiazem in guinea pig atria

| Ca-antagonists | pA₂ values (mean±S.E.) | Depolarized (A) | Normal (B) | Left atrium |
|----------------|------------------------|-----------------|------------|-------------|
| Nifedipine     | 5.90±0.16              | 5.90±0.16       |            |             |
| Verapamil      | 4.88±0.11              | 4.88±0.11       |            |             |
| Diltiazem      | 4.07±0.16              | 4.07±0.16       |            |             |

Depolarized: muscles were depolarized by high potassium Tyrode’s solution (22 mM K⁺). Normal: muscles were exposed to normal Tyrode’s solution. Each value is the mean±S.E. of 4 experiments.
became depolarized and inexcitable. Addition of isoproterenol (0.2 μM) under this condition, resulted in a restoration of contractility. The tension increased gradually over the succeeding five min to reach a steady value, which depended on the calcium concentration of the bathing media. After the establishment of a steady state, dose-response curves for calcium were obtained. Calcium antagonistic vasodilators were then added and cumulative dose-response curves of calcium were again obtained. As is exemplified in Fig. 2 for $1 \times 10^{-7}$ g/ml of diltiazem, a shift to the right of the dose-response curve occurred under the influence of the three vasodilators tested. From these recordings, $pA_2$ values were calculated as described above, and were 8.24 for nifedipine, 6.67 for verapamil and 6.57 for diltiazem (Table 1).

**Effects on the membrane potentials in normal Tyrode's solution**

Effects of verapamil and diltiazem on the electrophysiological characteristics of guinea pig atrial muscle fibers perfused with normal Tyrode's solution are shown in Fig. 3 and Tables 2 and 3. The effects of nifedipine on the electrical parameters are listed in Table 4.

![Fig. 2. Effect of diltiazem (DTZ) on the cumulative dose-response curves for calcium in the potassium depolarized guinea pig atria. Ordinate: Contractile tension (% of maximum). Abscissa: Concentration of CaCl$_2$ on log scale.](image)

![Fig. 3. Typical records of the effects of verapamil and diltiazem on the membrane potential of atrial muscle fibers placed in normal Tyrode's solution. Upper half of the recording (downward deflections) represents the first differential of the action potential ($V_{max}$).](image)
Changes in the resting potential and the amplitude of the action potential produced by verapamil were insignificant, whereas the maximum rate of rise \( (V_{\text{max}}) \) showed a tendency to decrease at \( 1.4 \times 10^{-5} \) g/ml of verapamil, and the duration of action potential measured at either 75% or 90% repolarization was lengthened significantly.

Diltiazem did not produce any change in the resting potential up to a dose of \( 5 \times 10^{-5} \) g/ml, but the amplitude of the action potential showed a tendency to decrease, albeit insignificantly.

### Table 2. Effects of verapamil on resting and action potentials of atrial muscle fibers

| Dose (g/ml) | Time (min) | A.P. (mV) | R.P. \(-\text{mV}\) | \( V_{\text{max}} \) (V/sec) | Duration of A.P. (msec) |
|-------------|------------|-----------|-------------------|-------------------------|------------------------|
|             |            |           |                   |                         | 75\%                   | 90\%                   |
| 4.5 \times 10^{-5} | 0            | 101.0 \pm 3.8 | 79.3 \pm 4.0     | 100.5 \pm 7.7     | 85.0 \pm 5.6           | 128.5 \pm 8.8          |
|              | 15          | 92.0 \pm 5.2  | 80.7 \pm 2.2     | 92.9 \pm 18.5     | 106.7 \pm 11.3        | 154.7 \pm 14.9         |
|              | 30          | 101.8 \pm 4.0 | 84.3 \pm 0.8     | 104.5 \pm 10.2    | 124.7 \pm 5.2**       | 179.0 \pm 3.5**        |
| 1.4 \times 10^{-5} | 0            | 102.8 \pm 4.9  | 84.5 \pm 0.5     | 94.8 \pm 15.9     | 124.7 \pm 5.2         | 179.0 \pm 3.5          |
|              | 15          | 97.3 \pm 5.7   | 83.8 \pm 3.7     | 74.8 \pm 8.4      | 147.3 \pm 13.2        | 208.8 \pm 13.9         |
|              | 30          | 96.8 \pm 7.0   | 86.5 \pm 3.8     | 74.7 \pm 9.6      | 153.0 \pm 5.3+        | 220.5 \pm 10.5*        |

Time: Time after addition of drug. A.P.: amplitude of action potential. R.P.: resting potential. \( V_{\text{max}} \): maximum rate of rise of action potential. Duration of A.P.: duration of action potential measured either at 75% repolarization or 90% repolarization. Each value is the mean \( \pm \) S.E. of 4 experiments. *\( p<0.05 \), **\( p<0.01 \)

### Table 3. Effects of diltiazem on the resting and action potentials of atrial muscle fibers

| Dose (g/ml) | Time (min) | A.P. (mV) | R.P. \(-\text{mV}\) | \( V_{\text{max}} \) (V/sec) | Duration of A.P. (msec) |
|-------------|------------|-----------|-------------------|-------------------------|------------------------|
|             |            |           |                   |                         | 75\%                   | 90\%                   |
| 1.0 \times 10^{-5} | 0            | 101.3 \pm 1.9 | 86.8 \pm 1.9     | 115.9 \pm 9.5     | 96.3 \pm 5.5           | 149.5 \pm 15.4         |
|              | 15          | 98.5 \pm 7.3  | 86.5 \pm 5.1     | 83.1 \pm 11.3     | 101.3 \pm 10.4        | 148.5 \pm 12.5         |
|              | 30          | 93.3 \pm 4.3  | 83.0 \pm 3.2     | 85.9 \pm 6.8*     | 115.8 \pm 8.0         | 172.0 \pm 7.2          |
| 5.0 \times 10^{-5} | 0            | 99.0 \pm 6.1  | 85.8 \pm 3.4     | 100.5 \pm 21.9    | 114.5 \pm 5.1         | 167.5 \pm 6.3          |
|              | 15          | 91.3 \pm 9.3   | 82.5 \pm 4.3     | 70.2 \pm 25.7     | 152.5 \pm 12.4*       | 206.5 \pm 6.4**        |
|              | 30          | 81.0 \pm 8.6   | 80.0 \pm 4.1     | 40.0 \pm 9.3*     | 160.3 \pm 8.8**       | 215.3 \pm 10.0**       |

Abbreviations are as in Table 2. Each value is the mean \( \pm \) S.E. of 4 experiments. *\( p<0.05 \), **\( p<0.01 \)

### Table 4. Effects of nifedipine on the resting and action potentials of atrial muscle fibers

| Dose (g/ml) | Time (min) | A.P. (mV) | R.P. \(-\text{mV}\) | \( V_{\text{max}} \) (V/sec) | Duration of A.P. (msec) |
|-------------|------------|-----------|-------------------|-------------------------|------------------------|
|             |            |           |                   |                         | 75\%                   | 90\%                   |
| 1.0 \times 10^{-5} | 0            | 101.5 \pm 2.6 | 85.8 \pm 2.5     | 113.0 \pm 4.4     | 86.0 \pm 10.5          | 138.5 \pm 10.3         |
|              | 15          | 100.7 \pm 3.8 | 81.3 \pm 3.0     | 108.1 \pm 3.3     | 84.7 \pm 4.5           | 135.3 \pm 7.4          |
|              | 30          | 102.8 \pm 2.5 | 83.8 \pm 2.9     | 115.3 \pm 6.6     | 89.8 \pm 4.8           | 143.5 \pm 6.8          |
| 1.0 \times 10^{-5} | 0            | 102.8 \pm 2.5 | 86.3 \pm 3.1     | 109.6 \pm 3.3     | 94.3 \pm 7.1           | 150.5 \pm 8.9          |
|              | 15          | 98.0 \pm 4.6  | 85.0 \pm 2.1     | 114.2 \pm 20.6    | 106.3 \pm 3.1         | 161.0 \pm 7.2          |
|              | 30          | 98.5 \pm 6.7  | 86.3 \pm 3.8     | 107.9 \pm 11.4    | 106.5 \pm 6.5         | 165.3 \pm 10.2         |

Abbreviations are as in Table 2. Each value is the mean \( \pm \) S.E. of 4 experiments.
The duration of action potential at either 75\% or 90\% repolarization was lengthened and $V_{\text{max}}$ was reduced at $1 \times 10^{-5}$ g/ml of diltiazem. Nifedipine did not produce any change in all the parameters of the action potential measured up to a dose of $3 \times 10^{-6}$ g/ml.

**Effects on calcium-dependent action potentials of the depolarized atrial muscle**

The atrial muscles, when exposed to Tyrode's solution containing 22 mM K$^+$ soon became unresponsive with reduction of the resting potential. Isoproterenol or aminophylline restored excitability of the depolarized muscle without inducing any further changes in the resting potential (Fig. 4, Table 5), and the amplitudes of the action potential evoked by these compounds were dependent on the calcium concentration of the bathing solution: $[Ca^{++}]_o$ varying by 29.0 mV (mean of 4 experiments) for a 10-fold change in $[Ca^{++}]_o$, in agreement with the value predicted by the Nernst equation (30 mV/decade). Three $\mu$M tetrodotoxin did not produce any change in the evoked action potentials, while these poten-

![Fig. 4. Restoration of action potentials in a depolarized atrial muscle by isoproterenol (Isp) and aminophylline (Amph). Atrial muscle was depolarized by high potassium (22 mM) Tyrode's solution.](image)

**TABLE 5.** Action potentials of guinea pig atria perfused with normal Tyrode's solution and with high potassium solution containing isoproterenol or aminophylline

|                      | AP amplitude (mV) | Resting potential (mV) | AP duration (msec) at 75\% repolarization |
|----------------------|-------------------|-----------------------|------------------------------------------|
| Normal Tyrode's solution | 105.7±2.4 (n=13) | 82.0±2.0 (n=13)       | 91.1±4.6 (n=12)                           |
| K-22 mM Tyrode's solution | — (unresponsive) | —                     | —                                         |
| K-22 mM Isoproterenol (0.3 $\mu$M) | 54.7±1.9 (n=17) | 42.2±0.6 (n=17)       | 55.5±1.6 (n=8)                            |
| K-22 mM Aminophylline (1.1 mM) | 58.0±2.1 (n=3)  | 40.7±1.2 (n=3)        | 70.3±4.2 (n=3)                            |

All values are mean±S.E. Number in parentheses indicates number of measurements.
tials were rapidly suppressed by Mn\(^{2+}\) (0.5 mM). Thus the action potentials can qualify as calcium-dependent potentials, as has already been demonstrated by Pappano (16).

Effects of nifedipine on the calcium dependent action potentials are shown in Fig. 5. While there were no significant changes after 1 x 10\(^{-9}\) g/ml of nifedipine, in doses above 3 x 10\(^{-9}\) g/ml, nifedipine produced a reduction of action potential amplitude which was not accompanied by a change in the resting potential and duration of the action potential. Diltiazem produced no change in the resting and action potentials of depolarized atria at a dose of 3 x 10\(^{-8}\) g/ml. There was a decrease in the amplitude after 1 x 10\(^{-7}\) g/ml (Fig. 5). However, the resting potential did not change even under this condition. Verapamil produced a reduction of the amplitude of action potentials evoked by aminophylline as well as those evoked by isoproterenol without producing any change in the resting potential (Fig. 6).

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**Fig. 5.** Effects of nifedipine and diltiazem on the action potential evoked by 0.2 μM of isoproterenol in atrial muscle depolarized by high potassium Tyrode's solution.

**Fig. 6.** Effects of verapamil on the action potential evoked by isoproterenol (Isp) or aminophylline (Amph) in atrial muscle depolarized by high potassium Tyrode's solution.
Quantitative comparison of calcium-antagonistic effects observed in the atrial action potential with those observed in the atrial contraction

As summarized in Fig. 7, the concentrations of calcium-antagonists which produce an inhibition of the Ca-dependent action potential and the Ca-dependent contraction in the depolarized guinea pig atria were almost the same.

In contrast, in normal Tyrode's solution, nifedipine caused ca. 50% reduction of the contractile force at $1 \times 10^{-6}$ g/ml, while this compound produced no change in $V_{\text{max}}$ and other parameters of membrane potential even at a concentration as high as $3 \times 10^{-6}$ g/ml. Diltiazem reduced the contractile force at $5 \times 10^{-5}$ g/ml, while the reduction of $V_{\text{max}}$ began at a smaller dose of this compound.

Verapamil produced a reduction of contractile force together with a slight reduction of $V_{\text{max}}$ at $1.4 \times 10^{-5}$ g/ml.

**DISCUSSION**

It is now well established that calcium plays an essential role in the contraction of various types of muscles. It has been independently reported that nifedipine, verapamil and diltiazem have a calcium antagonistic activity in both the cardiac (1-5, 9-15) and the smooth muscles (6-8).

However, there is apparently no documentation concerning the effects of several calcium-
antagonistic vasodilators in the same preparation to determine correlation of the changes
in the action potential with those in the contractility. Therefore, in the present study, a
quantitative comparison of the calcium-antagonistic effects of three representative calcium-
antagonistic vasodilators was undertaken to correlate the negative inotropic effects of these
compounds to their calcium antagonistic effects.

In the atrial muscle bathed in normal Tyrode’s solution, all the three vasodilators tested
produced a shift to the right of the dose-response curve for calcium and the order of potency
of this Ca-antagonistic action was nifedipine>verapamil>diltiazem. However, nifedipine
did not produce any change in the height and shape of action potential of atrial muscle even
in doses as high as $3 \times 10^{-6}$ g/ml. This finding is consistent with that of Fleckenstein et al.
(5), who noted that nifedipine exerted no influence on the membrane potential of the cat
papillary muscle even in doses at which the contractile response almost disappeared, but
is not in agreement with the data of Refsum (14) who demonstrated that nifedipine caused
a reduction of $V_{\text{max}}$ and a shortening of duration of the action potential in the isolated rat
left atrium. Unlike nifedipine, verapamil, in doses above $2.3 \times 10^{-5}$ g/ml, produced a re-
markable reduction of $V_{\text{max}}$ and a prolongation of duration of action potential, although this
compound produced no significant change in $V_{\text{max}}$ up to a dose of $1.4 \times 10^{-5}$ g/ml. Diltiazem
produced a definite reduction of $V_{\text{max}}$ and a prolongation of duration of action potential
in a dose of $5 \times 10^{-5}$ g/ml. The prolongation of the duration of action potential produced
by verapamil and diltiazem could have resulted from a decrease in potassium permeability.
If a K+-Ca++ exchange mechanism as postulated by Morad and Goldman (17) is operative
in guinea pig atria, the decreased potassium movement across the membrane could produce
a decrease in the influx of calcium. This possibility merits further exploration. The
definite decrease in $V_{\text{max}}$ observed after diltiazem and verapamil indicates that diltiazem
and, to a lesser degree verapamil, possess an inhibitory effect upon the Na carrier in addition
to their antagonistic effects towards calcium. Although Fleckenstein and his collaborators
demonstrated with a voltage-clamp method that verapamil and nifedipine inhibited the
transmembrane calcium-current during excitation without producing any change in the fast
sodium-current, it is now clear that verapamil and diltiazem can produce an inhibition of the
fast sodium-current, when used at a higher dosage level. This is in accordance with the
recent findings by Bayer et al. (15) and Nakajima et al. (13). However, the inhibition of
Na carrier produced by diltiazem and verapamil could not be the main cause of the negative
inotropic effect of these compounds, for Nakajima et al. (13) reported that the negative
inotropic effect of diltiazem was counteracted by addition of calcium, while the reduction
of the maximum rate of rise was not recovered. Furthermore, in the present experiments,
it was necessary to use a little higher concentration of verapamil to produce an inhibition
of sodium current than to produce a suppression of contraction of the normal polarized atria.
The fact that nifedipine produced a suppression of the contraction in normal Tyrode’s so-
lution, at a dose at which there was no inhibition of the sodium current, indicates that the
suppression of the contractile tension in the normal polarized atria can be induced without
any change in the fast sodium current.
Although it has been shown that the inward Ca\(^{2+}\) current is a significant contributing factor to the height and maintenance of the plateau phase of the action potential (18-22), it is by no means easy to evaluate the effects of any drug on the calcium current from the changes in the configuration of a normal cardiac action potential. Therefore, in the present study, the effects of vasodilators on the calcium current were studied using a calcium-dependent action potential of the depolarized guinea pig atria induced either by catecholamine or aminophylline. All the three vasodilators used suppressed the amplitude of Ca-dependent action potential and the doses at which suppression of the action potential occurred were in good parallel with the pA\(_2\) values for the suppression of contractile tension in the depolarized atria. This finding, if taken together with that of Thyrum (10) which showed that verapamil simultaneously depressed the electrical and mechanical activity of a field-stimulated guinea pig atrial preparation bathed in a high potassium Tyrode's solution containing catecholamine, suggests that the negative inotropic effect of these Ca-antagonists observed in depolarized atria may have resulted from a decrease in Ca influx during the action potential. This conclusion is consistent with the recent work by Watanabe and Besch (23) and Entman et al. (24) who demonstrated that reasonable doses of verapamil did not affect calcium binding or release by isolated sarcoplasmic reticulum fragments. Although there are reports (25-27) which suggest that verapamil has a \(\beta\)-blocking action, the antagonistic effect of verapamil towards Ca-dependent action potential cannot be explained solely by a blockade of \(\beta\)-receptor by this compound, since verapamil also inhibited the calcium-dependent action potential induced by aminophylline.

To determine the organ selectivity of calcium-antagonistic effects of these vasodilators, such effects of these compounds were studied in our laboratory using depolarized taenia coli (unpublished). The pA\(_2\) values obtained in the taenia coli were 9.43 for nifedipine, 7.36 for verapamil and 6.57 for diltiazem, indicating that the calcium-antagonistic effect of nifedipine was quite organ-selective, its potency in taenia coli being 15.5 times higher than in atria. Verapamil came next, while diltiazem had almost no selectivity, the ratio (smooth muscle/atria) being only 2.

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REFERENCES

1) FLECKENSTEIN, A., TRITTHART, H., FLECKENSTEIN, B., HERBST, A. AND GRÜN, G.: Pflügers Arch. 307, R 25 (1969)
2) FLECKENSTEIN, A., TRITTHART, H., FLECKENSTEIN, B., HERBST, A. AND GRÜN, G.: Arch. Pharmacoel. 264, 227 (1969)
3) FLECKENSTEIN, A., TRITTHART, H., DÖRING, H.J. AND BYON, K.Y.: Calcium and the heart. Edited by HARRIS, P. AND OPH, L., p. 135, Academic Press, London and New York (1971)
4) KOHLHARDT, M., BAYER, B., KRAUSE, H. AND FLECKENSTEIN, A.: Pflügers Arch. 335, 309 (1972)
5) FLECKENSTEIN, A., TRITTHART, H., DÖRING, H.J. AND BYON, K.Y.: Arzneim.-Forsch. 22, 22 (1972)
6) Grün, G., Fleckenstein, A. and Trithart, H.: Arch. Pharmacol. 264, 239 (1969)
7) Grün, G., Fleckenstein, A. and Byon, K.Y.: Arzneim.-Forsch. 21, 1585 (1971)
8) Grün, G. and Fleckenstein, A.: Arzneim.-Forsch. 22, 334 (1972)
9) Trithart, H., Volkmann, R., Weiss, R. and Fleckenstein, A.: Arch. Pharmacol. 280, 239 (1973)
10) Thyrum, P.T.: J. Pharmacol. exp. Ther. 188, 166 (1974)
11) Cranefield, P.F., Aronson, R.S. and Wit, A.L.: Circulation Res. 34, 204 (1974)
12) Shigenobu, K., Schneider, J.A. and Sperelakis, N.: J. Pharmacol. exp. Ther. 190, 280 (1974)
13) Nakajima, H., Hoshiyama, M., Yamasita, K. and Kiyomoto, A.: Japan. J. Pharmacol. 25, 383 (1975)
14) Resum, H.: Acta pharmacol. toxicol. 37, 329 (1975)
15) Bayer, R., Kaluschf, D., Kaufmann, R. and Mannhold, R.: Arch. Pharmacol. 290, 81 (1975)
16) Pappano, A.J.: Circulation Res. 27, 379 (1970)
17) Morad, M. and Goldman, Y.: Prog. Biophys. Mol. Biol. 27, 257 (1973)
18) Carmeliet, E. and Vereecke, J.: Pfliigers Arch. 313, 300 (1969)
19) Beecher, G.W. and Ruffner, H.: J. Physiol. 207, 191 (1970)
20) Matsubara, T. and Matsuda, K.: Japan. J. Physiol. 19, 814 (1969)
21) Ochi, R. and Trautwein, W.: Pfliigers Arch. 232, 187 (1971)
22) New, W. and Trautwein, W.: Pfliigers Arch. 334, 24 (1972)
23) Watanabe, A.M. and Besch, H.R.: J. Pharmacol. exp. Ther. 191, 241 (1974)
24) Lehn, M.L., Allen, J.C., Boren, E.P., Gillett, P.C., Wallick, E.T. and Schwartz, A.: J. Mol. cell Cardiol. 4, 681 (1972)
25) Melville, K.J. and Benfey, B.G.: Canad. J. Physiol. Pharmacol. 43, 339 (1965)
26) Nayler, W.G., McInnis, L., Swann, J.B., Price, J.M., Carson, V., Race, D. and Lowl.T.E.: J. Pharmacol. exp. Ther. 161, 247 (1968)
27) Haas, H. and Busch, E.: Arzneim.-Forsch. 17, 257 (1967)