Cardiovascular effects of diltiazem, a calcium antagonist, on isolated heart muscle preparations and in anaesthetized and pithed rats

J.P. BOUDOT, I. CAVERO & A.G. ROACH

SYNTHELABO, L.E.R.S., Department of Biology, Cardiovascular Unit, 58, rue de la Glaciere, 75621 Paris, France.

Diltiazem (d-cis-isomer of 3-acetoxy-2,3-dihydro-5-(2-(dimethylamine)-ethyl)-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4-(5H)-one.HCl) has been shown to produce myorelaxant effects in several tissues such as the isolated ileum contracted with various spasmogens (Nagao, Sato, Iwasawa, Takada, Ishida, Nakajima & Kiyomoto, 1972) and canine coronary arteries depolarized with potassium (Nagao, Ikeo & Sato, 1977).

In man, the compound has been reported to decrease cardiac work, an effect which is beneficial in the relief and prevention of angina (Kusukawa, Kinoshita, Shimono, Tomonaga & Hoshino, 1977).

The present study describes the cardiovascular effects of diltiazem in several *in vitro* and *in vivo* preparations.

In the rat spontaneously beating right atrium diltiazem (10 μM), in contrast to (±)-propranolol, produced a non-competitive antagonism of isoprenaline positive chronotropic responses.

In the electrically paced (100 beats/min) rat heart, perfused with Tyrode solution (5 ml/min) using the technique of Langendorff, the dose of diltiazem exerting 50% inhibition of the baseline contractile force was 6.7 μM. The same effect was produced by decreasing the extracellular calcium concentration from 2.2 to 0.62 mM. The negative inotropic effect of 10.0 μM diltiazem was partially antagonized (70–80%) by doubling the normal calcium ion concentration of the Tyrode solution.

In paced (60 beats/min) rabbit heart (prepared as the rat heart) diltiazem (1.0 μM; perfusion flow: 20 ml/min) decreased left ventricular pressure by 50% and perfusion pressure by 12% at the end of the 10 min infusion. The duration (at 0 and −60 mV) of the left ventricular epicardial action potential (AP) and the resting potential were not significantly changed by diltiazem. However, the rate of rise of the AP as well as the overshoot was significantly decreased.

Finally, in the intact pentobarbital anaesthetized rat, diltiazem (156 $\mu g\ kg^{-1}\ min^{-1}$ for 60 min) induced a significant and sustained fall in carotid blood pressure and heart rate. In the pithed rat, the same dose of diltiazem reduced significantly the increases in blood pressure and heart rate elicited by i.v. noradrenaline, 5-hydroxytryptamine and angiotensin II as well as electrical stimulation of the spinal cord.

These results suggest that diltiazem may affect the excitation–contraction coupling. An effect on intracellular free calcium, essential for contractile processes, is probable since the phase of the ventricular action potential depending on the calcium membrane permeability was not significantly modified. This conclusion is consistent with the results obtained by Nakajima, Hoshiyama, Yamashita & Kiyomoto (1975) using the isolated guinea-pig papillary and ventricular muscles exposed to diltiazem (1.0 μM).

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The sensitivity of superfused bioassay tissues

E.V. BAGSHAW
(introduced by A. BENNETT)

Department of Surgery, King's College Hospital Medical School, London SE5 8RX.

Some authors (e.g. Ferreira & De Sousa Costa, 1976) have overestimated the sensitivity of superfused tissues by assuming that the response is proportional to the total dose. The present work confirms the statement by Gaddum (1953) that the response is proportional to the concentration, and shows in addition that the contact time can be important. Strips of rat gastric fundus were set up at 37°C in oil baths and superfused by Krebs solution as described by Ferreira & De Sousa Costa (1976), using a variable-rate peristaltic pump (LKB 2120 Peripex). The Krebs solution contained antagonists (Gilmore, Vane & Wyllie, 1968)

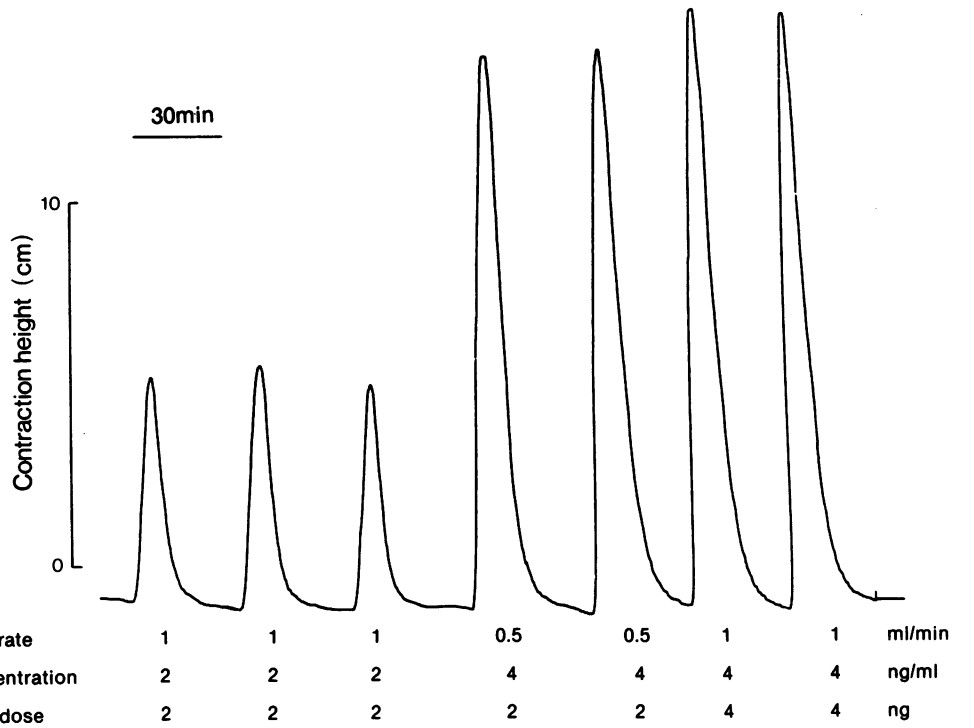

Figure 1 The response of the rat stomach strip to PGE_2 (1 min contact) depended on agonist concentration and (not shown) contact time. Responses were similar using the same concentration at different flow rates and total dosage.

and indomethacin (1 μg/ml). Prostaglandin E_2 (PGE_2) was applied by removing the fine (1 mm i.d.) inlet tube from the Krebs solution for 10 s to form an air bubble, immersing the tube in a PG solution, and then returning it to the Krebs solution after allowing another air gap to prevent dilution in the tubing. There were at least 10 experiments of each type described below.

With a constant contact time (1 min) and total dose (4 ng PGE_2) the response was proportional to the concentration (0.4–8 ng/ml, 1.0–0.05 ml/min; Figure 1).

With a constant concentration and contact time (1 ng/ml PGE_2 for 1 min), flow rates of 0.1–1.0 ml/min produced similar responses (Figure 1).

With a constant concentration and total dose (1 ng/ml, 0.25 ng PGE_2) the response was proportional to the contact time (15–120 s). The contact time is important probably because the rat fundus responds slowly.

The threshold sensitivity was 50 pg/ml, but the threshold total dose was 5 pg. Ferreira & De Sousa Costa (1976) also obtained a sensitivity of 5 pg, but this was in 0.1 ml and the concentration was 50 pg/ml. By calculating as total dose, slowing the flow rate gives an illusion of increased sensitivity.

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