PROLONGED PROTECTIVE EFFECT OF
THE CALCIUM ANTAGONIST ANIPAMIL ON
THE ISCHEMIC REPERFUSED
RABBIT MYOCARDIUM:
COMPARISON WITH VERAPAMIL

SUMMARY. To assess whether pretreatment with the calcium antagonist anipamil protects the heart against ischemic and reperfusion damage and to establish how long the protection persists after cessation of the therapy, rabbits were injected subcutaneously twice daily for 5 days with 2 mg/kg body weight of this drug. The heart was then isolated 2, 6, or 12 hours after the last injection and was perfused by the Langendorff technique during a control period and 90 minutes of total ischemia (37°C), followed by 30 minutes of reperfusion. Diastolic and developed pressure was monitored; coronary effluent was collected and assayed for creatine phosphokinase (CPK); mitochondria were harvested and assayed for respiratory activity, ATP production, and calcium content; and tissue concentration of adenosine triphosphate (ATP) and creatine phosphate were determined. The data obtained with anipamil were compared with those obtained with verapamil administered to the rabbit at the same dose and following the same procedure.

Pretreatment with anipamil induced a negative inotropic effect under normoxic conditions; reduced the rate and extent of depletion of ATP and creatine phosphate during ischemia, with an incomplete restoration of the nucleotides after reperfusion; maintained mitochondrial function and calcium homeostasis during ischemia and reperfusion; reduced the rate of CPK release; and improved the recovery of ventricular function on reperfusion. The protective effects of anipamil persisted for as long as 12 hours after the last administration. In contrast, the protective and negative inotropic effects of verapamil were no longer apparent in heart isolated 6 or 12 hours after the last dose of the drug.

It is concluded that anipamil pretreatment provides a protection against some of the deleterious effects of myocardial ischemia and reperfusion and that this effect is substantially longer than that of verapamil. The protective effect of anipamil (like that of verapamil) is probably secondary to a reduction of the rate of ATP hydrolysis during ischemia, although alternative mechanisms of action cannot be excluded.

KEY WORDS. anipamil, verapamil, calcium antagonist, ischemia, reperfusion, cardioprotection

Cellular damage due to myocardial ischemia followed by reflow has been found to be associated with a 10- to 20-fold increase in intracellular calcium concentration [1]. An increase of intracellular calcium is thought to potentiate ischemic cell injury by increasing energy utilization and decreasing ATP production [2]. These considerations have stimulated, during the last few years, a growing interest in the possibility of reducing ischemic injury with agents commonly referred to as calcium antagonists [3-5].

To account for the clinical usefulness of these drugs in the treatment of ischemic heart disease, we have to consider that they may affect the functioning of cardiac and smooth-muscle cells and the cells of pacemaker, nodal, and conducting tissues [6-9]. Although most of the studies performed in animal models have shown calcium antagonists to be effective in reducing myocardial ischemia, the results of the available clinical trials have been disappointing [10-14]. The failure of these trials to provide unequivocal evidence of protection has emphasized the need to use these substances as prophylactic agents [15, 16]. Indeed, when used prophylactically, they have been shown to be protective [17-21]. Therefore, the timing of treatment and the duration of cardiac protection are critical factors [7, 15, 16].

The following experiments were undertaken to establish whether the new calcium antagonist anipamil is able to reduce some of the deleterious effects caused by ischemia and reperfusion in the isolated and Langendorff-perfused rabbit's heart and how long the protection persists after cessation of therapy. As
anipamil is a long-acting derivative of verapamil [22],
its effects have been compared with those of verapamil,
which, in this study, was used as a pharmacologic
control.

Myocardial damage was measured in terms of
mechanical performance, CPK release, determination
of isolated mitochondrial function, mitochondrial
calcium overload, and the rate and extent of ATP and
creatine phosphate depletion during ischemia and
reperfusion.

Methods

Animals

Adult, male, New Zealand white rabbits (2.5-3.0 kg
body weight) were used. The control rabbits received 2
ml/kg of 0.9% sodium chloride solution or ethanolic
solution injected subcutaneously twice daily. The
other rabbits (treated groups) were injected sub-
cutaneously twice daily with either 2 mg/kg of
anipamil (Knoll AG) in ethanolic solution or 2 mg/kg of
verapamil (Knoll AG) dissolved in the same volume of
sodium chloride solution. Treatment was given for 5 or
6 days to ensure effective blocking of the slow calcium
channels. The animals received the last injection
either 2, 6, or 12 hours before the experiment.

The doses of anipamil and verapamil selected for
this study were employed after an initial period of test-
ing several different doses. Doses of verapamil and
anipamil of 6 mg/kg and 8 mg/kg twice daily killed the
animals. Doses of either drug below 1 mg/kg did not
exert any noticeable effect on the left ventricular pres-
sure or heart rate of the isolated hearts. The dose
regimen selected for this study is the same as that used
in other studies, at least for verapamil, where it pro-
vided plasma levels that are comparable with those
used clinically [2]. Similar data for anipamil are not
available. All the treated animals survived and there
were no exclusions.

Perfusion of the Hearts

The rabbits were stunned by a blow on the neck. The
hearts were rapidly excised and perfused by the non-
recirculating Langendorff technique, using a modified
Krebs Henseleit buffer solution containing 1.5 mM of
calcium, as previously described [23, 24]. The hearts
were then paced at 180 beats/min using suprathreshold
rectangular pulses of 1.0 ms duration [23] and perfused
either under control-aerobic condition (coronary flow
25 ± 1.7 ml/min) or made totally ischemic (abolishing
coronary flow) for 10, 30, or 90 minutes. In separate
groups of experiments, the hearts, after 90 minutes of
ischemia, were reperfused for 30 minutes at a coronary
flow of 25 ml/min. Left ventricular wall temperature
was maintained at 36°C to 37°C, irrespective of cor-

Left Ventricular Pressure

To obtain an isovolumetrically beating preparation, a
fluid-filled balloon was inserted into the left ventricle
via the left atrium. The intraventricular balloon was
connected by a fluid-filled polyethylene catheter to a
Statham pressure transducer (P23Db) for the deter-
mination of left ventricular pressure, as previously de-
scribed [23].

Coronary Effluent Analysis

During aerobic perfusion and postischemic reperfu-
sion, the coronary effluent was collected by timed

collection into chilled glass vials and assayed, on

the same day, for creatine phosphokinase activity (CPK).
CPK activity was measured spectrophotometrically
following the method described by Oliver [25].

Mitochondrial Studies

1. Isolation of the mitochondria: Mitochondria were
isolated at the end of each perfusion by differential
centrifugation, as previously described [23, 26].
Two different isolation media were used. The
mitochondria required for oxygen consumption
studies were isolated in the medium described by
Sordhal et al. [27], containing KCl 180 mM, EDTA
10 mM, and BSA 0.5%. The mitochondria used for
the determination of endogenous calcium and of
ATP production were extracted in a medium con-
taining sucrose 250 mM and ruthenium red 5 μM
[28]. Ruthenium red, an inhibitor of mitochondrial
calcium uptake, was included to prevent calcium
accumulation during the isolation procedure.

2. Oxygen consumption measurements: Rates of oxy-
gen consumption were monitored polarographically
at 25°C using a Clark-type electrode. 1.25 mg/ml of
mitochondrial protein was suspended in 2 ml of a
solution containing sucrose 250 mM, KH₂PO₄ 3
mM, EDTA 0.5 mM, and glutamate 3 mM, pH 7.4
adjusted with Tris buffer, and allowed to equili-
brate for 1 minute. State 3 respiration was initiated
by adding ADP 0.5 mM. Mitochondrial function
was assessed in terms of RCI and QO₂, where QO₂ is
n atoms of oxygen used per milligram of mitochon-
drial protein in response to the addition of ADP.
RCI (respiratory control index) is the ratio of oxy-