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Antibiotics newborn
heart sympathetic nervous system
inotropic agent

Response of the Neonatal Heart to a New Inotropic Agent, RO2-2985 (X537A)

PAUL C. GILLETTE,^{1,2} RONALD G. MUNSON, ROBERT M. LEWIS, AND ARNOLD SCHWARTZ

Department of Cell Biophysics, Division of Myocardial Biology, and Department of Pediatrics, Section of Cardiology, Baylor College of Medicine and the Fondren-Brown Cardiovascular Research and Training Center, The Methodist Hospital, Houston, Texas, U.S.A

Extract

The ionophore RO2-2985 did not produce a positive inotropic response in 1-day-old canine isolated ventricle. A gradual increase in inotropic response was seen with age. Isolated atria, however, exhibited a positive inotropic response at birth (50% increased dF/dt), which became progressively greater with age (100% increase in dF/dt at 15 days of age). In the neonatal heart *in situ* there was a positive inotropic response in 1-day-old puppies (40% increase in left ventricular dF/dt) with progressively greater responses with age (135% increase in left ventricular dF/dt at 11 days of age). There was a positive chronotropic (75-125% increase) response to RO2-2985 at all ages studied. The drug elevated systemic arterial pressure (150% increase in mean arterial pressure) to a similar degree in all ages studied. RO2-2985 depressed total calcium binding by both neonatal and adult isolated cardiac sarcoplasmic reticulum approximately 50%.

Speculation

Although the exact mechanism of action of RO2-2985 on the heart remains unproven, the temporal association of the increase in ventricular and atrial sympathetic nerve endings with development reported by previous authors and the increase in response to RO2-2985 and tyramine suggest that the drug might act in part by releasing a humoral substance. The discrepancy between *in situ* and isolated studies tends to support this view. A more complete delineation of all subcellular fractions of the developing heart will be necessary to clarify the differential actions of this and other drugs on the developing heart. RO2-2985 may be of value in the treatment of low cardiac output states in neonates as well as adults, but specific testing in neonates will be necessary.

The antibiotic ionophore RO2-2985 (Hofmann-LaRoche), in exerting a significant effect on the cardiovascular system,

prompted the suggestion of potential therapeutic usefulness (2, 3, 10, 18, 19). Studies on isolated cardiac tissues have shown an increase in contractile force after administration of RO2-2985 (2, 3, 10, 18, 19). This has been confirmed in intact preparations (2, 3, 18). The heart rate and systemic blood pressure were also increased. Although the exact mechanism of action of RO2-2985 remains unknown, it is known to enhance transport of both mono- and divalent cations as well as biologically active amines across membranes and to promote a calcium proton exchange (6, 14). The drug also decreases calcium binding and uptake by isolated cardiac sarcoplasmic reticulum (SR) and rapidly releases calcium that was previously bound to the SR (4, 5, 15).

All of the published work to date on RO2-2985 has employed adult cardiac tissue. It was the purpose of this study to evaluate the effects of RO2-2985 on the neonatal canine heart in order to obtain further data concerning mechanism of action.

MATERIALS AND METHODS

Four preparations were used: (1) isolated perfused ventricle, (2) isolated atrium, (3) intact *in situ* heart, and (4) isolated sarcoplasmic reticulum. All were obtained from mongrel puppies of both sexes whose exact age was known and who had been kept with their mothers until used. RO2-2985 was dissolved in benzyl alcohol, anhydrous alcohol, and propylene glycol. The vehicle alone was used for a control in all experiments. The order of administration of RO2-2985 and tyramine was alternated to decrease the effect of tachyphylaxis. RO2-2985 and tyramine were always given before other drugs. The puppies from each litter were used in series (*i.e.*, for a six-puppy litter, two were studied at each of three ages).

ISOLATED VENTRICULAR PREPARATION

The animal was killed by cervical dislocation and the heart was rapidly removed and suspended on a constant flow device (18) at 37° and perfused via the aortic root with Krebs solution with the following millimolar concentration: NaCl 118, KCl 4.7, CaCl₂ 2.5, NaHCO₃ 25, MgCl₂ 0.5, NaH₂PO₄ 1.0, and glucose 11.1, pH 7.30–7.40. The Krebs solution was continuously bubbled with 95% O₂:5% CO₂.

The atria were removed, the atrioventricular node crushed, and pacing accomplished by wires placed in the right ventricle at a rate of 120 cpm. The atrioventricular valves were excised and the pulmonary artery opened. A Walton-Brodie strain gauge arch (20) was sutured to the free wall of the left ventricle.

Krebs solution prewarmed to 37° was perfused at a constant pressure of 20 mm Hg by an occlusive peristaltic pump. Drugs were administered by changing from one reservoir to another without interrupting flow. Developed force as well as its first derivative with respect to time (dF/dt) were continuously recorded on a Grass model 7 polygraph. This equipment and solution are the same as previously described for the cat heart from this laboratory (18). Four preparations were studied at each age.

ISOLATED ATRIA

After the heart had been perfused, as in the preceding section, the atria were removed and a strip of left atrium was attached by suture to a Statham force transducer (350 ohms) and tension adjusted for maximum force of contraction. Drugs were then added to the solution and mixed by a constant stream of 95% O₂:5% CO₂ (pH 7.30–7.40). The temperature of the bath was maintained at 37°. Force and dF/dt were continuously recorded on the Grass polygraph. Four preparations were studied in each age range.

IN SITU HEART

The animals were anesthetized with sodium pentobarbital (25 mg/kg intraperitoneal) and respiration controlled with a Harvard model 606 respirator pump for small animals by way of a

tracheostomy. Temperature was continuously monitored and controlled at 37° by electric heating pads. Arterial blood gases were maintained in the normal range by adjusting the respirator. The carotid artery was cannulated with a short piece of polyethylene tubing directly attached to a Statham P23db strain gauge. A second polyethylene catheter was advanced well into the internal jugular vein for infusion of drugs.

The chest was opened and the heart cradled in the pericardium. A strain gauge arch was sutured to the free wall of the left ventricle. The preparation was covered with a sheet of polyethylene to prevent excessive evaporation. Electrocardiogram, central aortic blood pressure, left ventricular force, and dF/dt were continuously recorded on a Brush model 200 recorder.

Two preparations were studied at 1 day of age, four preparations at 2.5 days of age, five at 4 days of age, and two at 11 days of age.

ISOLATED CARDIAC SARCOPLASMIC RETICULUM

The sarcoplasmic reticulum was isolated as described previously by this laboratory (8). Maternal SR was isolated simultaneously to serve as a control. A dual-beam spectrophotometric method using murexide as a calcium indicator was used to measure calcium "binding" (in the absence of oxalate) and "uptake" (presence of oxalate) (4, 5, 8).

RESULTS

ISOLATED VENTRICULAR PREPARATION

The inotropic response of isolated ventricles from 1, 10, and 15 days of age to RO2-2985 was evaluated and compared with their response to tyramine, a known catecholamine releaser. There was no increase in the force of contraction of isolated ventricles up to 2 days of age with either RO2-2985 or tyramine (Fig. 1). Older ventricles responded, and the amount of increase in force of contraction and dF/dt was proportional to age (Figs. 1 and 2). The ventricles which would not respond to RO2-2985 or tyramine did increase their force of contraction after administration of norepinephrine, isoproterenol, or ouabain (data not shown).

ISOLATED ATRIA

There was a positive inotropic response to RO2-2985 and tyramine by the atrium of even the youngest animals studied (Fig. 3). The increased force of contraction to the same concentration was progressively greater at increasing ages (Figs. 2 and 3). The

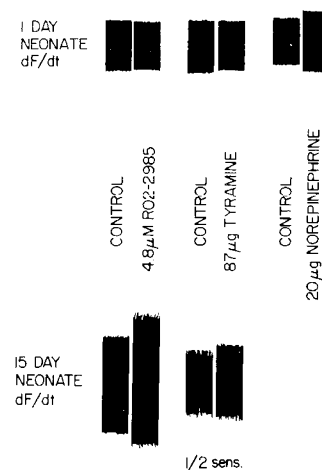


Fig. 1. Maximum response of the isolated perfused developing canine ventricular preparation to RO2-2985 and tyramine at the 1st day and 15th day of age. The original tracing of the first derivative of force with respect to time is presented.

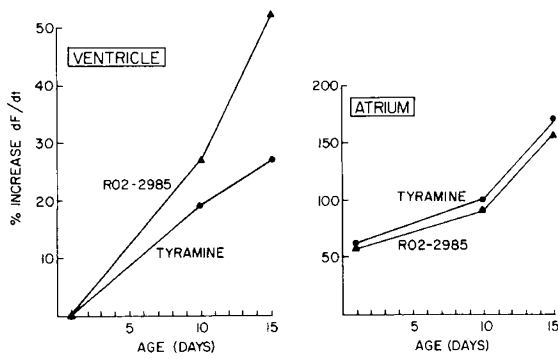


Fig. 2. Graph of the relationship of the maximum positive inotropic response of the developing isolated ventricle and atrium to RO2-2985 and tyramine.

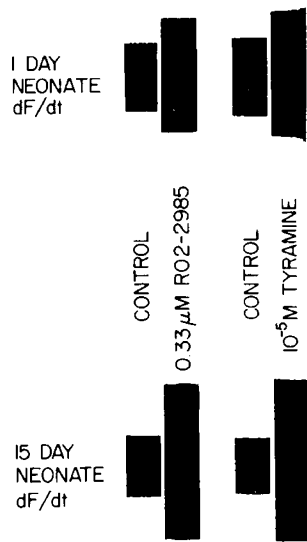


Fig. 3. Maximum positive inotropic response of the developing atrium to RO2-2985 and tyramine using dF/dt as an index of contractility.

magnitude of the atrial response was greater at all ages than that of the ventricle.

IN SITU HEART

RO2-2985 and tyramine both exerted a positive inotropic response on the *in situ* left ventricle at 2 days of age (Fig. 4). The response increased with age, reaching essentially adult levels by 12 days of age (Figs. 5 and 6). RO2-2985 produced a 100% increase in heart rate in puppies from 1-12 days of age. There was also a 100% increase in mean aortic blood pressure in response to RO2-2985 in all ages studied.

In one 7-day-old puppy, a single injection of RO2-2985 produced a 40% increase in LVdF/dt and a 50% increase in central aortic pressure. After this effect had disappeared and the animal was intentionally put into hypovolemic hypotension (shock) by withdrawing blood until a mean arterial pressure of 25 mm Hg was reached, an equal injection of RO2-2985 increased LVdF/dt 230% and central aortic pressure 150% (Fig. 7).

ISOLATED CARDIAC SARCOPLASMIC RETICULUM

RO2-2985 depressed calcium binding and uptake of the isolated sarcoplasmic reticulum of 1-, 3-, and 7-day-old neonatal canines to a similar degree as the maternal control. A whole litter of puppies (6-10) had to be used for each preparation. Each preparation was tested in triplicate and the results averaged. A representative experiment is shown in Figure 8. No difference was noted between the activity of the neonates and maternal controls.

DISCUSSION

The cardiovascular response in the early postnatal period to RO2-2985 is markedly different than in adult animals. In the intact preparations this difference was manifested in two ways. A 100% increase in LVdF/dt was seen in adult animals after injection of 1 mg/kg of RO2-2985, whereas in 1- and 2.5-day-old puppies, less than 50% increase was found. By 12 days of age the positive inotropic response reached the adult level. The isolated ventricle showed no increase in contractile force to RO2-2985 before 3 days of age. This discrepancy between the isolated and *in situ* preparations was confirmed by studying puppies from the same litter on the same day using both methods. There was no response by the

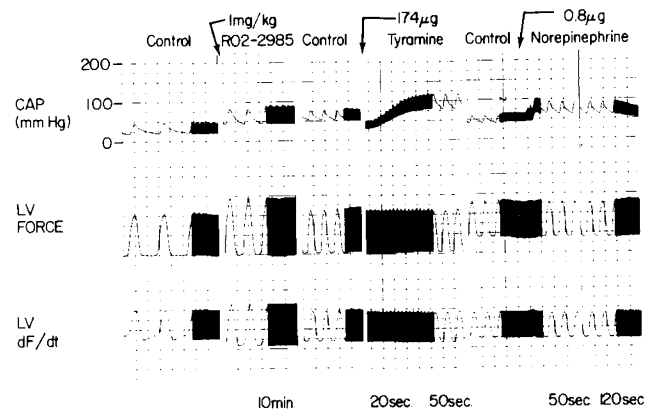


Fig. 4. Hemodynamic effects of RO2-2985 on a 2.5-day-old puppy. From top to bottom: the central aortic pressure (CAP), the left ventricular force and its first derivative. From left to right: control period, maximum effect of RO2-2985 (10 min), return towards control, effect of tyramine, return towards control, effect of norepinephrine.

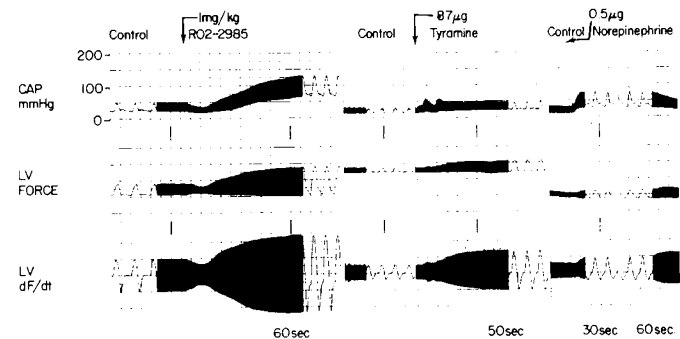


Fig. 5. Hemodynamic effects of RO2-2985 on a 12-day-old puppy. Relationship of events the same as in Figure 4.

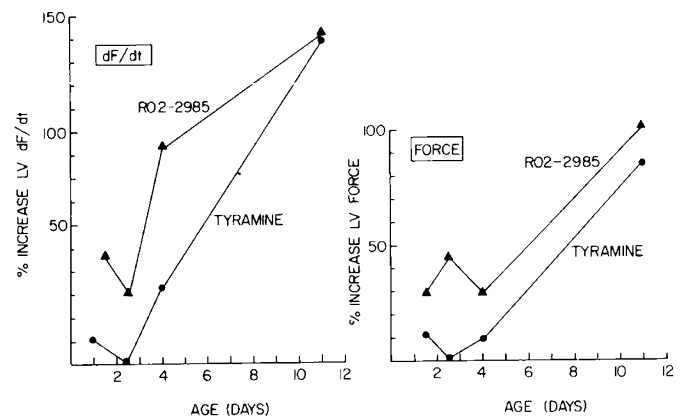


Fig. 6. Graph of the changes with development of the inotropic response to RO2-2985 of the *in situ* heart using LVdF/dt and LV force as indices of contractility.

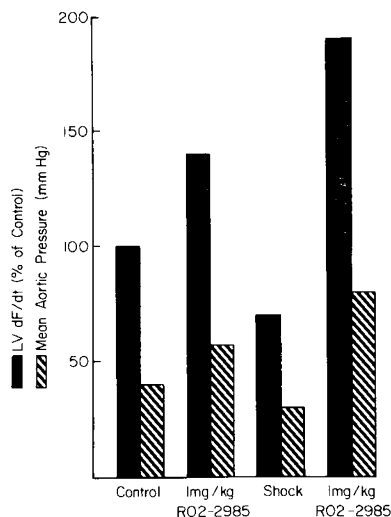


Fig. 7. Changes in LVdF/dt and aortic pressure of a 7-day-old puppy exposed to RO2-2985 before and after induction of hypovolemic hypotension ("shock").

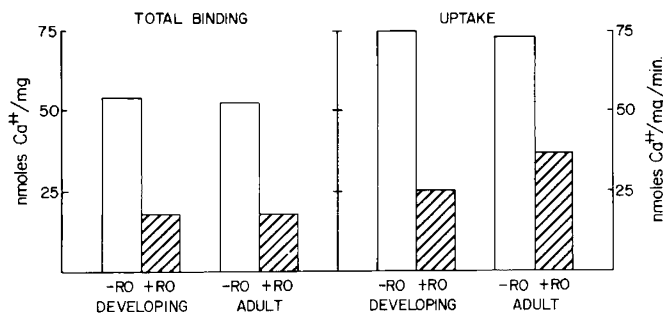


Fig. 8. Effect of RO2-2985 (RO) on calcium binding and uptake of neonatal and adult isolated cardiac sarcoplasmic reticulum. No differential effect of the drug was found.

isolated 1.5-day-old ventricle to a concentration of RO2-2985 which stimulated older ventricles. The *in situ* ventricle, however, exhibited a 48% increase in dF/dt. This discrepancy could be explained by either the lack of sympathetic innervation of the isolated ventricle or the absence of some circulating factor. Several investigators have pointed out that the development of the sympathetic nervous system is not complete at birth in several species, including dogs, cats, sheep, rabbits, and swine (7, 12).

We postulate that part of the action of RO2-2985 is in some way mediated by sympathetic nerve endings. This could be accomplished by either the ionophore directly "transporting" the hormone out of the nerve ending, by transporting calcium and/or sodium into it, or by promoting a calcium proton exchange, as has been suggested for vasopressin release (13). It is of interest that a specific calcium and magnesium ionophore, A23187, has no cardiovascular effect (18). This would explain several aspects of our study. The sympathetic nerve endings are plentiful in the atria at birth, and RO2-2985 and tyramine produced a marked positive inotropic response from birth. The sympathetic nerve endings innervate the ventricles progressively over about the same time period as the increase in response to RO2-2985 and tyramine. The discrepancy between *in situ* and isolated ventricles could also be due to a "circulating factor(s)" released from the adrenals by RO2-2985. The transporting ability of RO2-2985 for biologically active amines is also in support of this contention (14). The fact that reserpine preparations do not exhibit a positive inotropic response to RO2-2985 (18) supports but does not prove our contention, inasmuch as reserpine has many actions (17). The failure of propranolol to block the inotropic response to RO2-2985 (18) is against a mechanism of action directly involving the

β -receptor; however, propranolol is less effective in blocking the action of endogenously released catecholamines than exogenously administered drugs (9). It is also known that RO2-2985 has marked effects on the peripheral vasculature in adult dogs. It increases renal, coronary, and iliac blood flow significantly without any significant change in mesenteric blood flow (11, 16, 18). Thus, the cardiac output may be redistributed.

Since the timetable of developments of the cardiac sympathetic nervous system is not known for the human, the actions of the drug may be very different in the neonate vs the adult. If this drug acts by releasing a hormone or some other substance from sympathetic nerve endings, its usefulness in patients with chronic congestive heart failure may be limited, since catecholamines are depleted in this state (1).

SUMMARY

The neonatal canine heart has been found to be less responsive to the inotropic drug RO2-2985 than the adult heart. The inotropic response to the drug develops with increasing age, reaching adult levels at about 2 weeks of age. This parallel development of response to the drug and previously known completion of sympathetic innervation to the heart suggests that the drug may interact with the sympathetic nervous system.

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22. Requests for reprints should be addressed to: P. C. Gillette, M.D., Department of Cell Biophysics, Baylor College of Medicine, 1200 Moursund Ave., Houston, Tex. 77025 (USA).

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Amylase	inorganic phosphate
calcium	parotid saliva
cystic fibrosis	potassium
flow rate	sodium

Parotid Gland Function in Children with Cystic Fibrosis and Child Control Subjects

JEANETTE BLOMFIELD,^{1,3,1} ANNE R. RUSH, HELEN M. ALLARS, AND JOHN M. BROWN

Children's Medical Research Foundation and Cystic Fibrosis Clinic, Royal Alexandra Hospital for Children, Sydney, New South Wales, Australia

Extract

Parotid saliva was collected from 22 children with cystic fibrosis (CF) and 21 control children. Stimulation was by 0.1 ml 5% citric acid on the tongue every 15 sec over a period of 10 min, and samples were collected for 2-min intervals. Stimulated saliva showed a lag period of low amylase release, and an activated period of higher amylase release. The major difference between CF and child control parotid saliva was in the elevated calcium concentrations in CF subjects. Flow rate and amylase, although higher in the CF group, were not raised to a statistically significant level. There were higher mean values for sodium and inorganic phosphate in CF children but statistical significance was minimal. Potassium values were almost identical in CF and control saliva. In the activated parotid saliva samples of both control and CF subjects there were positive correlations between flow rate and calcium, flow rate and sodium, amylase and calcium, and sodium and calcium, and negative correlations between sodium and potassium. The parotid function test has no value as a diagnostic test for cystic fibrosis, as even calcium values show too much overlap with control saliva to be of diagnostic worth.

Speculation

Higher calcium values in parotid saliva of CF children are likely to cause increased formation of insoluble calcium-phosphate-protein complexes within the acinar lumina and small ducts of the parotid gland and increased deposition of dental calculus on tooth surfaces adjacent to the openings of the parotid ducts.

In a previous study (5), parotid saliva collected during a short term (4-min) stimulation had shown elevated protein, amylase, and calcium concentrations in cystic fibrosis (CF) parotid saliva compared with control saliva. The higher protein concentrations were associated with colloidal separation of sparingly soluble protein on cooling the saliva to room temperature. Subsequently, parotid saliva with high calcium concentrations has been shown to form turbid calcium-phosphate-protein complexes on standing at body temperature (1, 2). It has been suggested that this sparingly soluble material might be causing obstruction in parotid

glands of CF patients (22) and excessive dental calculus on their teeth (26).

Previous comparisons of CF and control parotid saliva have produced conflicting results, showing that, in CF parotid saliva, flow rate is increased (18) or not different (4, 6, 13), protein is increased (3, 4) or not (16, 25), amylase is elevated (3, 4) or not (15), calcium is raised (3, 4, 6, 18, 25) or not (16, 18), sodium is higher (4, 6, 12, 18) or not (16), inorganic phosphate is increased (4, 6, 16), and potassium is not different from controls (4, 6, 12, 16, 18).

However, studies by Dawes (8, 9) of parotid saliva of normal subjects have shown that stimulated saliva takes approximately 3 min to change from a resting to an activated state, that variation of degree of sucking lozenges influences flow rate, that flow rate influences concentrations of protein and electrolytes, and that the duration of stimulation also influences concentrations of protein and calcium. Therefore, many of the previous studies of CF saliva are difficult to interpret. Thus variation among both CF and control children in rates of chewing chicle (6), or sucking lozenges (16, 18) or citric acid crystals (12) would affect results randomly. In other studies, collection times were variable among subjects (6), were not stated (15, 25), or were short (3, 4).

In the present study, the stimulus was constant and operator controlled, and the effects of duration of stimulation were assessed by 2-min sampling over a 10-min period. Correlations between flow rate, amylase, and inorganic components were determined in the activated saliva collected between 4 and 10 min after commencing stimulation.

SUBJECTS AND METHODS

Parotid saliva was collected from 22 children with cystic fibrosis (13 male and 9 female, with a mean age of 9.2 years and range of 6-13 years) who were attending the Cystic Fibrosis Clinic at the Royal Alexandra Hospital for Children as outpatients. There were 21 control children (13 male and 8 female, with a mean age of 9.2 years and range of 5-13 years), who were children of members of staff and their friends. Informed consent was obtained for all subjects.