Chronotropic Responses of Human Heart Tissue Cultures

By T. D. Chang and G. R. Cumming

ABSTRACT

Three presumptive cardiac tissues from aborted human embryos which ranged in length from 2 mm to 4 mm and in age from 53 days to 71 days were maintained in culture beating rhythmically for 18-88 days. Medium renewal, temperature increases (from 31° to 38°C), and pH increases (from 6.92 to 7.75) raised the pulsation rate. Excess potassium stopped the pulsation. Sympathomimetic drugs increased the pulsation rate, and this action was blocked by the beta-receptor inhibitor, propranolol, suggesting the presence of functioning beta-receptor sites in the heart cells by the end of the second month of development.

KEY WORDS culture medium temperature pH inorganic ions sympathomimetic drugs pulsation rate beta-receptor inhibitor beta-receptor site

Although numerous in vitro studies have been done on beating cardiac tissues and cells from experimental animals (1-4), there has been a surprising paucity of similar work on human material (5-8). In the course of cytogenetic studies of human spontaneous abortions, we found cells maintaining a rhythmic beat in three tissue cultures. Various experiments were performed to test the effects of physical and chemical agents on the pulsation rates of these three tissues.

Methods

All three presumptive cardiac tissues were from aborted human embryos contained in intact sacs. The embryos were abnormally small (2—4 mm in length); gestation histories indicated that their ovulation ages were about 53-71 days (expected crown-to-rump length about 30-50 mm).

The embryos were cut up into pieces approximately 1 mm² and planted in plasma clots in Leighton tubes. The culture medium (H597, Connaught Laboratories, enriched with 15% fetal calf serum, containing penicillin 100 units/ml, streptomycin 200 µg/ml, and chloramphenicol 5 µg/ml) was changed after 5 days and every other day thereafter. The tissues were examined for pulsation in the Leighton tube on a heated stage, and motion pictures were taken for documentation.

To subject the heart tissues to a certain temperature level, that part of the Leighton tube containing the tissue was submerged in a water bath at the desired temperature. The pH of the medium was adjusted by adding isotonic sodium bicarbonate or hydrochloric acid, using phenol red as an indicator. In testing the tissues with different chemicals, care was taken when the tissue was transferred from the control medium to the test medium to maintain the same temperature and pH.

For testing the effect of temperature, each of the tissues was subjected to three temperature levels at pH 7.4 for 5 minutes before observations were recorded. For testing the effect of pH, tissue 1 and tissue 2 were placed in culture media with pH values varying from 6.92 to 7.75 for 5 minutes at a time at 37°C.

The original concentrations of Na⁺, K⁺, Ca²⁺, and Mg²⁺ in the medium were 118.10 mEq/liter, 4.37 mEq/liter, 1.90 mEq/liter, and 1.22 mEq/liter, respectively. To test for the effects of individual inorganic ions, the concentration of Na⁺, K⁺, Ca²⁺, or Mg²⁺ in the medium was increased by 6.9 mEq/liter. The four ions were added in the form of NaCl, KCl, CaCl₂, and MgCl₂•6H₂O, respectively. In each test involving a different ion, the heart tissue was first treated with control medium for 5-15 minutes at 37°C and pH 7.4.
RESPONSES OF HEART CULTURES

The effects of varying concentrations of epinephrine, norepinephrine, isoprenaline, propranolol, phentolamine, atropine, acetylcholine, and combinations of propranolol and epinephrine were extensively studied. The tissue on each occasion was first tested in control medium for 15 minutes at 37°C and pH 7.4. The tissue was left in the test medium for 10 minutes. Usually the pulsation rate became constant after a few minutes, and only the rates at 10 minutes are reported.

Results

The pulsations of tissues 1, 2, and 3 lasted 77, 88, and 18 days, respectively. Their initial pulsation rates at 37°C and pH 7.4 were 94, 36, and 65 beats/min, respectively, and the rates declined steadily during the course of the culture period. Beating ceased when the tissues were overgrown by fibroblasts. Sectioning of the tissues at this stage failed to reveal any striated muscle cells. Chromosome studies of the fibroblasts showed that tissue 1 was a male trisomic for chromosome 16, tissue 2 was a female trisomic for chromosome 16, and tissue 3 was a normal male.

In tissue 2, on day 31, when the pulsation rate of the main mass had declined to 14 beats/min, a tiny cluster of cells on the edge of the tissue was seen pulsating independently at 36 beats/min, the initial rate of the whole tissue.

EFFECT OF CHANGE OF MEDIUM

The pulsation rate of tissue 1 was 61-76 beats/min when the medium was changed every other day and 94-100 beats/min when the medium was changed daily.

EFFECT OF TEMPERATURE

Pulsation rate increased progressively as the temperature of the medium was raised from 31° to 38°C in all three tissues (Fig. 1). Q10 was estimated to be 2.1 for tissue 1 and 3.4 for tissue 2. On one occasion, when tissue 1 was accidentally cooled down to room temperature (27°C), the beating stopped, but it resumed after reincubation.

EFFECT OF pH OF MEDIUM

The pulsation rate increased when the pH of the medium was raised (Fig. 2). The experiments on the effects of pH, inorganic salts, and various pharmacological agents reported below were conducted in the later stages of the development of the cultures; this probably accounts for the absolute pulsation...
EFFECTS OF INORGANIC IONS

Excess Na\(^+\) and Mg\(^{2+}\) in the medium slowed the pulsation rate 1-6 beats/min, but excess K\(^+\) stopped the pulsations in tissues 1 and 2. Irregular beating was noticed early during the exposure of tissue 2 to the excess K\(^+\). Excess Ca\(^{2+}\) increased the pulsation rate in tissue 1 and slowed it in tissue 2 by 10-20 beats/min.

EFFECTS OF PHARMACOLOGICAL AGENTS

The effects of three different sympathomimetic drugs on pulsation rates in tissue 1 are shown in Figure 3. Epinephrine produced a maximal pulsation rate at a lower concentration than did isoprenaline and norepinephrine. The peak rate of 90 beats/min was the same for each agent. Control rates were quickly restored after exposure to isoprenaline and epinephrine but not after exposure to norepinephrine.

Figure 4 shows similar results in tissue 2. The pulsation rate reached a plateau after exposure to epinephrine and isoprenaline at 10\(^{-8}\) g/ml. The control rate was slower at the time of exposure to norepinephrine, and the peak rate was lower and was not attained until the drug was present at a concentration of 10\(^{-6}\) g/ml. Atropine produced a gradual decrease in pulsation rate from 36 beats/min to 12 beats/min. Acetylcholine had little effect on the rate until standstill occurred at a drug concentration of 10\(^{-4}\) g/ml.

Figure 5 illustrates the partial blocking action of propranolol on the rate increase produced by epinephrine in tissue 2. Epinephrine alone increased the pulsation rate from 31 beats/min to 64 beats/min, and propranolol alone caused a slow reduction in pulsation rate and then arrested the pulsation at a drug concentration of 10\(^{-7}\) g/ml. The pulsation rate was 61 beats/min in culture medium with epinephrine at 10\(^{-7}\) g/ml. Maintaining the same epinephrine content and adding propranolol at 10\(^{-8}\)-10\(^{-4}\) g/ml slowed the rate to 36 beats/min, almost the control value. On a separate occasion, the pulsation rate was 19

![Figure 3](http://circres.ahajournals.org/)

**Figure 3**

Effects of three sympathomimetic drugs on the pulsation rates of tissue 1. Age of tissue was between 55 and 58 days.
Effects of three different sympathomimetic drugs and of atropine and acetylcholine on the pulsation rates of tissue 2. Age of tissue was between 23 and 25 days for all tests except that with acetylcholine; this test was made on day 36.

Blocking action of propranolol on the increase in pulsation rate caused by epinephrine in tissue 2. Age of tissue was 25 days. Note that the upper two curves represent epinephrine treatment alone and treatment with epinephrine at $10^{-7}$ g/ml in combination with varying concentrations of propranolol. The lower two curves represent propranolol treatment alone and treatment with propranolol at $10^{-8}$ g/ml in combination with varying concentrations of epinephrine.
beats/min in culture medium with propranolol at \(10^{-8}\) g/ml. Increasing the concentration of epinephrine in the above medium raised the pulsation rate to a peak of 42 beats/min, well below the peak rate observed with epinephrine alone. Phentolamine, an alpha-receptor inhibitor, had no effect on the pulsation rate and failed to inhibit the response to epinephrine.

**Discussion**

By using abortion materials in the classical type of explant culture, it is possible to obtain beating human heart tissues in vitro for experimental studies. From our results, it seems to be advisable to use tiny embryos (2-4 mm long) contained in intact sacs. We have tried, without success, to culture heart fragments from larger fetuses (2-10 cm long, crown to rump) contained in intact sacs and from at least one tiny embryo (3.5 mm long) contained in a broken sac. The method of our explant culture in plasma clots also appears to be usable for the present purpose. Pulsation of the tissue can be maintained for up to 88 days, considerably longer than the 4 days of Morosow (5), the 14 days of Timofejewsky and Benewolenskaja (6), and the 36 days of Höfer (7) in their human embryonic heart explant cultures.

Höfer (7) observed in his heart culture from a 6- to 8-week-old human embryo that a smaller central portion beat in its own rhythm faster than the rest. Garry and Townsend (8) reported that in their cultures from a 13-week-old human embryo, the ventricular preparation had a slower pulsation rate than did the sinoatrial preparation, giving a ratio of 1 to 3.6. It is possible that, in our tissue 2, there was a larger ventricular portion and that the fast-beating cluster was of sinoatrial origin. As the tissue aged, the latter no longer effectively led the pace of the former. Although it has been estimated that human hearts first pulsate at or about the twenty-second day of development (9), our results suggest either that the intrinsic rate dominance between the ventricular tissue and the sinoatrial tissue is established before the age of our embryo (53 days) or that the main mass of tissue had slowed down for lack of essential nutrients or other causes related to the long duration of its existence in culture (10).

When the effects of some of the agents were tested on more than one heart tissue, the responses differed considerably in intensity. This probably reflects variation in the condition or the constitution, or both, of the tissues we used. It may also be pointed out that our tissue 2 stopped beating on day 7 under normal conditions and was revived only with a brief treatment of epinephrine. Thus, tissue 2 could conceivably have been less responsive to certain agents than tissue 1.

The increase in pulsation rate caused by the daily renewal of the medium was not due to the difference in the pH of the medium, since the medium had the same pH before and after it was changed. Possibly factors such as supply of food and oxygen were involved.

The results of our experiments on temperature effects are similar to those of Höfer (7) and Garry and Townsend (8).

The results on the effects of inorganic ions seem to be in general agreement with those on chick embryonic heart cultures in which excess Na⁺, K⁺, Ca²⁺, or Mg²⁺ in the culture medium impaired normal cardiac contractility (1). Excess K⁺ stopped the pulsation, possibly by depolarizing the cell membrane (11).

The expected chronotropic response of these human heart tissue cultures to sympathomimetic drugs suggests the presence of functioning beta-receptor sites. This was confirmed when the response to epinephrine was inhibited by the beta-receptor blocking agent, propranolol. Human embryonic heart cells are clearly capable of responding to catecholamines by the end of the second month of development.

Some studies on chick embryonic heart cells grown in tissue cultures have shown that the pulsation rate is unresponsive to catecholamine stimulation (12), but others have shown that the pulsations are accelerated (13). The failure to respond to catecholamines raises the question of whether nerve elements are required for a catecholamine response; it has
been previously demonstrated that intact chick hearts are stimulated by epinephrine prior to the establishment of innervation (14). It is possible that the trypsin dispersion of some tissue cultures interferes with the receptor sites, accounting for the controversial results.

In the present study, the pulsations showed little change when the tissue was exposed to low concentrations of acetylcholine, but they ceased when the concentration was increased to $10^{-4}$ g/ml. Intact embryonic chick hearts at an age before innervation are arrested by acetylcholine at a concentration of $10^{-6}$ g/ml (14). Thus embryonic heart cells would appear to be no different from mature cardiac tissue in their response to acetylcholine.

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References

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