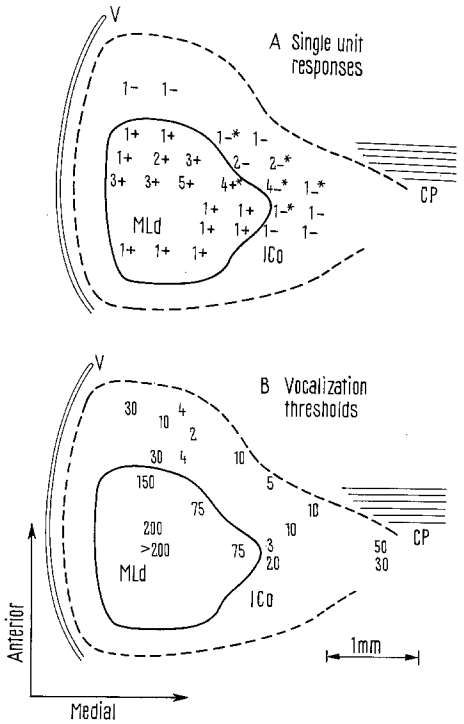
least 0.4 mm beyond the depth yielding the lowest threshold. 4 tracks were run through the right optic lobe of each subject at intervals of 1.0–1.5 mm, 2 tracks to a sagital plane.



Dorsal view of the *nucleus mesencephalicus lateralis pars dorsalis* (MLd) and the *nucleus intercollicularis* (ICo) depicting (A) single unit responses to acoustic stimulation and (B) vocalization thresholds for electrical brain stimulation. ——— border of MLd, ····· border of ICo, ═══ lateral border of ventriculus (V), ▒▒▒ *commissura posterior* (CP). A) Each + or − symbol respectively indicates the presence or absence of acoustic units at that approximate position. The number of electrode penetrations within a given area is indicated by the accompanying arabic numeral. Sites that yielded 'acoustic' units within the *formatio reticularis lateralis* (ventral to the MLd and ICo) are marked with asterisk. B) The position of each number indicates the approximate position of a stimulating electrode track. The magnitude of the number indicates the lowest vocalization-elicitation threshold in μA within the MLd or the ICo.

The results from 9 male quail are summarized in Figure 1, B. All tracks that entered the medial anterior portions of the ICo produced vocalization at low threshold. Different sites within the ICo of the same subject sometimes yielded different types of vocalizations. This is in accord with previous results demonstrating a degree of neuroanatomical separation within the ICo of regions from which different natural calls can be elicited[1].

The threshold for eliciting vocalization from within the medial and anterior portions of the ICo were quite low when compared with stimulation within the MLd (electrodes penetrated the MLd in 5 out of 9 subjects). The threshold for vocalization within the MLd tended to vary with distance of the track from medial and anterior portions of the ICo. This suggests that vocalization elicited by stimulation within the MLd resulted from current spread to these portions of the ICo. Concurrent work by NEWMAN[4] with the redwinged blackbird, using evoked potentials as a criterion for responsiveness to acoustic stimuli, also shows that electrical stimulation of the MLd and other midbrain auditory areas does not elicit vocalization at current strength levels that elicit vocalization in the ICo. These results indicate that the ICo, and not the MLd, probably plays some role in the production of natural vocalization. Whether the fact that the MLd and the ICo are adjacent is of functional significance remains to be determined[9,10].

Zusammenfassung. Das Gebiet innerhalb der *torus semicircularis* eines Vogels mit niedriger Schwelle zur Vokalisierung der Natur sehr ähnelnder Rufe ist räumlich von demjenigen Gebiet getrennt, das auf akustische Reize reagiert.

L. M. POTASH

Department of Psychology, University of Alberta, Edmonton 7 (Canada), 13 January 1970.

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Response to Adrenaline, Acetylcholine and Change of Contraction Frequency in Early Human Foetal Hearts

The foetal cardiovascular system is considered to be under control of nervous and hormonal influences during the latter part of gestation[1]. However, little information is available concerning the time course of development of receptors to autonomic transmitter substances in man. Adrenergic innervation cannot be detected histochemically in the human foetal heart ventricles before the 12th week of gestation[2]. The present report describes observations concerning inotropic and chronotropic responses to adrenaline and acetylcholine in 2 human foetal heart preparations of 9 and 10 week gestational age. The positive inotropic effect of adrenaline was compared to that evoked by an increase in contraction rate. Recordings of electrical transmembrane potentials were performed in the same hearts to obtain data concerning the electrophysiological maturity of the myocardium at this gestational age.

Material and methods. 2 foetal hearts were obtained at legal evacuation of uterus in the 9th and 10th week of gestation, respectively. The crown-rump length of both

[1] N. S. ASSALI, *Biology of Gestation* (Academic Press, London/New York 1968), vol. 2.

[2] G. GENNSER and CH. OWMAN, unpublished observation.

foetuses was 30 mm, the wet weight of the heart preparation was 46 and 24 mg, respectively. The hearts were immediately isolated and brought into a modified Ringer's solution of the following composition (mM): NaCl 120, $NaHCO_3$ 20, KCl 4.0, $CaCl_2$ 2.0, NaH_2PO_4 1.5, $MgSO_4$ 1.5, dextrose 3.3. The pH of the solution was kept at 7.3 by continuous vigorous bubbling with 95% O_2 and 5% CO_2. For electrophysiological measurements the hearts were mounted in a small thermostated bath. The right atrium and ventricle were opened to provide better access to, and enhance oxygenation of, the surface-perfused myocardium according to a pattern described earlier[3]. Bipolar glass electrodes with Ag/AgCl wires in 0.9% NaCl-agar were used for extracellular recording of electrogram. One bipolar electrode was placed in the coronary sinus region, the other immediately below the right atrio-ventricular border. Atrioventricular conduction time was measured from the peak of the atrial to the peak of the ventricular electrogram wave. For intracellular determination of transmembrane potentials microelectrodes with resistances of 10–20 $M\Omega$ and a double-sided cathode-follower were used. Ventricular strips were cut from the atrioventricular border to the apex and mounted in a thermostated bath for mechanical recordings. One end of the preparation was attached to a tension transducer, RCA 5734, for recording of isometric contractions. The signals from the cathode follower and from the tension transducer were displayed on an Elema-Schönander Mingograph or a Tectronix 502A oscilloscope. Stimulation was achieved by means of current pulses of 2 msec duration with stimulus intensity 50% above the threshold value. All experiments were performed at 37 °C.

Adrenaline bitartrate and acetylcholine chloride in freshly prepared stock solutions were added to the perfusion fluid to give the final concentrations of adrenaline or acetylcholine indicated below.

Results. During perfusion with a solution containing 0.8×10^{-7} g/ml adrenaline, an increase in contraction rate from 140 to 184 beats/min occurred in the spontaneously beating 10-week heart (Figure 1). Adrenaline in this concentration also shortened the atrio-ventricular conduction time recorded in the external electrogram from 144 to 127 msec (mean of 5 measurements). The heart rate remained above the control value during 5 min adrenaline exposure and this response could be repeated after washing the heart with Ringer's solution. Acetylcholine in concentrations of 0.8×10^{-7} to 4×10^{-6} g/ml induced a lowering of the contraction frequency and a gradual lengthening of the atrioventricular conduction time. The chronotropic effect of 4×10^{-6} g/ml acetylcholine is seen in Figure 2. At the time of the last point in the figure an atrio-ventricular block occurred. Adrenaline caused a positive inotropic response in a ventricle strip (11 mg wet weight) from the 9-week heart. When stimulated at a constant frequency of 90 stimulations/min, after addition of adrenaline (10^{-7} g/ml) the preparation increased its peak tension to 4 times the pre-adrenaline reading (Figure 3). Acetylcholine (3×10^{-7} g/ml) did not cause any detectable inotropic change.

As illustrated in Figure 4 the foetal ventricle strip responded to a rise in the stimulation rate with an increase in peak isometric tension. At stimulation rates above 104 stimulations/min a conduction block occurred within the preparation. Even, at a contraction frequency near the optimal for isometric twitch tension, addition of adrenaline could elicite a considerable increase in mechanical output of the preparation (Figure 4; note different tension scales in Figures 3 and 4).

No detailed electrophysiological study was performed but resting membrane potentials around — 70 mV were recorded in several cells from the strip of the right ventricle. One action potential and the corresponding isometric twitch curve is shown in Figure 5. The resting membrane potential in this cell was — 74 mV. No diastolic depolarizations were seen in any of the ventricle cells impaled.

Discussion. The present findings indicate that the adrenergic receptor mechanism in the human myocardium is present early in ontogenesis, before the establishment of the adrenergic innervation. Positive inotropic adre-

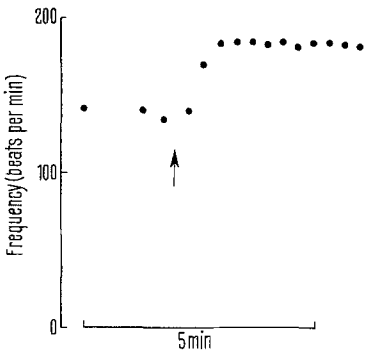

Fig. 1. Effect of adrenaline (0.8×10^{-7} g/ml) on spontaneous contraction rate of an isolated heart from a 10-week human foetus. Temperature in this and following figures 37 °C.

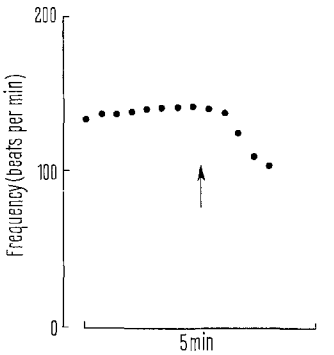

Fig. 2. Chronotropic effect of acetylcholine (4×10^{-6} g/ml) on same heart preparation as in Figure 1.

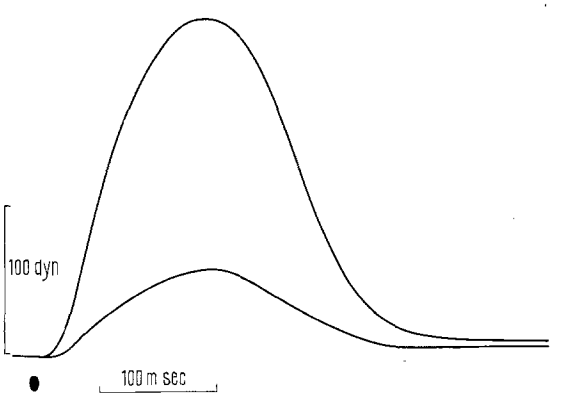

Fig. 3. Inotropic effect of adrenaline (10^{-7} g/ml; ventricle strip from 9-week foetus). Isometric tension before (lower curve) and after (upper curve) addition of adrenaline. Stimulation rate 90 stimulations/min. Stimulation artefact visible. Redrawn from oscilloscope tracings.

[3] G. GENNSER and E. NILSSON, Acta physiol. scand., in press.

nergic effects have been observed also in non-innervated foetal chick myocardium[4]. The chronotropic effects of adrenaline and acetylcholine recorded in the human foetal heart agree with results on embryonic non-innervated myocardium from rat[5] and chick[6]. It is of interest to note that embryonic chick hearts not only respond to adrenaline and acetylcholine before innervation has occurred, but also liberate acetylcholine during electrical stimulation[7]. The origin and function of this acetylcholine, however, is not known.

The pronounced inotropic response to adrenaline observed in the human foetal ventricle might be considered consistent with the absence of competition between the adrenergic receptors and the catecholamine uptake mechanism in nerve terminals[8]. This suggestion is supported by the report that the response of the sparsely innervated foetal lamb myocardium to adrenaline is stronger than that of corresponding adult myocardium[9]. In the latter study it was also shown that the adrenergic response of foetal myocardium, in contrast to adult, could not be further potentiated by cocaine[9]. A positive inotropic

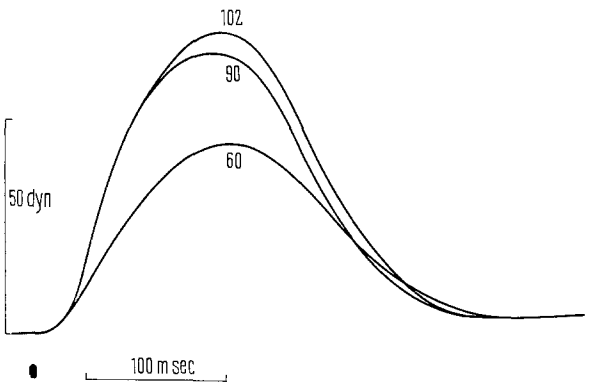

Fig. 4. Isometric tension curves at different stimulation frequencies. Same preparation as in Figure 3. Stimulation artefact visible. Numbers indicate contraction rate in beats/min. Redrawn from oscilloscope tracings.

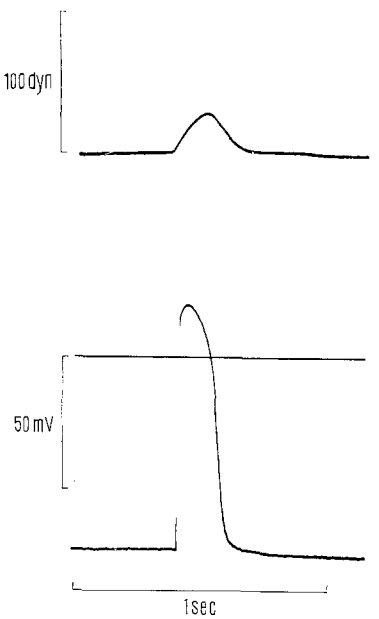

Fig. 5. Simultaneously recorded isometric tension curve (upper record) and action potential (lower record). Same preparation as in Figure 3. Stimulation frequency 26 stimulations/min. The horizontal line in lower record represents zero potential.

effect due to an increase in contraction rate has been found in embryonal hearts from chick as early as 3 days and 21 h of age[10], i.e. before innervation of the heart has occurred[11]. Isolated chick embryonal myocardial cells in culture have also been shown to exhibit a frequency-dependant inotropic response[12]. The present study reveals that in human myocardium at a stage prior to adrenergic innervation, an increase in contraction frequency exerts a positive inotropic effect similar to that found in human papillary muscle later in gestation[13].

The amplitude of the resting membrane potential found in the present investigation, e.g. — 74 mV in Figure 5, is only slighty less than that recorded in human heart cells both from midterm foetus[3] and from adult atrium[14,15] and adult ventricle[14,16]. The recorded amplitude of the resting membrane potential seems to indicate a high effectiveness of a cation pump mechanism already at this foetal age (25% of gestational length). This is at variance with the finding of an increase in amplitude of the resting membrane potential in rat foetal heart during the latter half of gestation, from — 30 mV to — 80 mV[17]. Diastolic depolarizations indicating latent pacemaker activity have been demonstrated both in atria and ventricles in foetal rat[17] and also in atria from human foetal hearts[3]. In the foetal rat heart diastolic depolarizations disappear gradually first in the ventricles, then in the atria[17]. Therefore, a stable resting membrane potential, as is observed in ventricle cells in the present study, seems to suggest a relatively high degree of maturity already at the 10th week of gestation[18].

Zusammenfassung. Adrenalin rief einen positiv chronotropen und inotropen, Acetylcholin einen negativ chronotropen Herzeffekt bei zwei menschlichen Foeten von 30 mm Scheitel-Steiss-Länge hervor. Ein Treppenphänomen wurde in Ventrikelstreifen und stabile Membranpotentiale (— 70 mV) in einzelnen Ventrikelzellen nachgewiesen.

G. Gennser and E. Nilsson

Department of Obstetrics and Gynecology at the General Hospital, S-21401 Malmö (Sweden), and Department of Pharmacology, University of Lund, S-22362 Lund (Sweden), 15 May 1970.

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