the second chromatographic separation and subjected to thin layer chromatography as described for the food. A spot corresponding to HVA was found in the pre-glucose urine but was not detectable in the post glucose urine.

An O-acetyl methyl ester derivative[7] was prepared from HVA added to urine and treated by the above extraction and chromatographic procedures. From chromatography of this derivative in an F & M Biomedical Model 400 gas chromatograph it was determined that the recovery of HVA was about 60% of the added material.

It was determined, therefore, that HVA excretion is markedly reduced upon glucose feeding, although HVA is known to be a metabolic product of dopamine and is believed to be of endogenous origin.

Discussion. The importance of diet in influencing chemicals found in the body fluids and particularly in urine has long been recognized. However, the possible complexity of this relationship has often been ignored. It has frequently been assumed that, if a product disappears from the urine when potential food sources have been removed, this product was in fact contained in the food. In this study we have demonstrated that the concentration of a known metabolic product, HVA, can be strikingly reduced by dietary modification even though its presence in the food cannot be demonstrated by 2 different extraction techniques. The apparent decrement in HVA in the urine could have occurred because of an increase in conjugation in the presence of a large increase in glucose[8], a decrease in available precursors or a shift in urinary pH resulting in diminished renal clearance[9]. 1 or more of these factors may also be involved in the findings of Von Studnitz in regard to DMPEA[1]. These alternative possibilities appear to offer at least as good an explanation as that DMPEA is ingested in food.

Zusammenfassung. Reine Glukose-Diät führt zur Abnahme der Ausscheidung von Homovanillinsäure im Rattenharn. Die Abnahme trat auch dann ein, wenn keinerlei präformierte Homovanillinsäure im Rattenfutter nachgewiesen werden konnte.

A. J. Friedhoff and J. Coupet

Center for Study of Psychotic Disorders, Department of Psychiatry and Neurology, New York University, School of Medicine, New York City (N.Y. 10016, USA), 20th January 1967.

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Effect of Locally Applied Acetylcholine on the Embryonic Cardiac Action Potential

Acetylcholine (ACh) is known to cause an increase in P_K in mammalian sinoatrial (SA) and atrial fibres[1] and has therefore been employed in attempts to analyse the components of the cardiac action potential. By use of locally applied ACh, atrial and perinodal action potentials have been shown to consist of an initial, fast component (spike) followed by a prolonged, slow component (plateau), whereas potentials from SA and AV (atrioventricular) nodal cells contain only the slow component[2]. These findings provide the basis for considering the development of the cardiac action potential as consisting of 2 functional parts: a fast process representing the P_{Na} system[1,3] and a slow process involving, in part, a decrease in P_K[1,4]. Other studies have considered the dual nature of the amphibian cardiac action potential from several points of view[5].

The present study was undertaken, first, to reveal the presence or absence of the 2-component system in transmembrane potentials recorded from embryonic chick heart cells and, second, to relate the findings to the development of electrical activity in the embryonic heart.

The procedure for preparing the embryonic chick hearts (14–19 days) for microelectrode analysis is described in detail elsewhere[6]. Acetylcholine (60 μg/ml) is applied to the preparation by local ejection of a modified Tyrode solution through a micropipette (tip diameter 50 μ) situated very close to the recording microelectrode. This technique was adapted from similar studies of the adult mammalian heart[2] and enables simultaneous intracellular recording during local applications of acetylcholine.

The Figure (A, B, C, D) shows the effect of this procedure on 4 types of cardiac action potentials. In Figure A, the atrial action potential is shortened in duration, but the peak potential is unaltered or slightly decreased. Figure B shows the response of a transmembrane action potential from the atrial margin of the AV ring, in which acetylcholine produces a marked decrease in peak potential as well as a shortened action potential. Although not shown in the Figure, ventricular cells do not noticeably respond to the local applications of ACh. However, the action potential duration of cells in the ventricular portion of the AV valve is still somewhat sensitive to acetylcholine (Figure C). It is of interest to note the progressive delay in the response of the valve cell to the driving stimulus (not visible on record). A progressive delay in AV transmission due to acetylcholine is known to occur in the embryonic chick heart[7]. Thus, the response shown in Figure C is most probably caused by ACh, as it affected cells of both the AV ring and AV valve. The depolarizing

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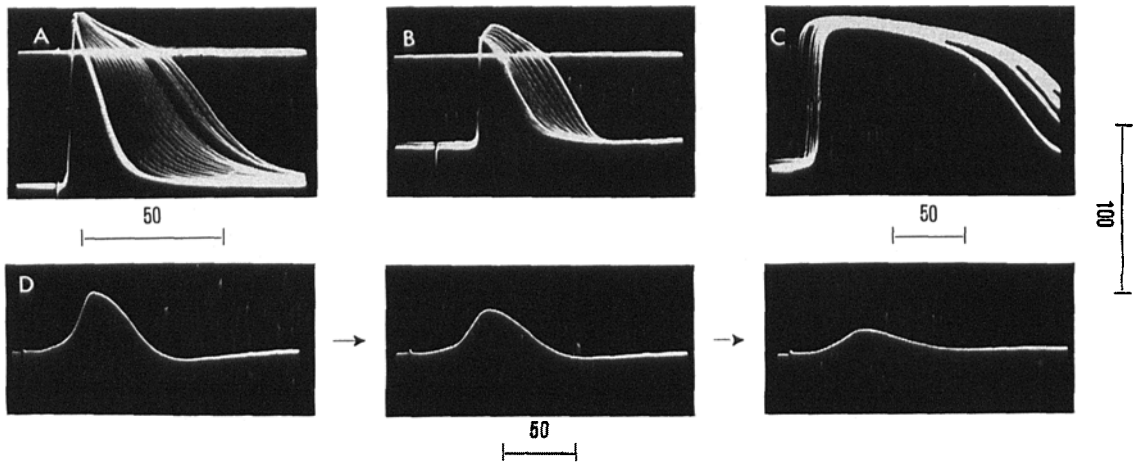

Effect of locally applied acetylcholine on transmembrane action potentials from the embryonic chick heart. (A) right atrium, (B) atrial margin of AV ring, (C) AV valve (ventricular portion), (D) middle portion of AV ring. (A)–(C) are superimposed records of consecutive sweeps. Vertical bar = 100 mv; horizontal bars = 50 msec.

effect observed in Figure C is most probably attributable to the active movement of the muscular AV valve, causing the electrode to be dislodged from the cell. In Figure D, the consecutive frames depict the manner in which cells from the middle of the AV ring respond to ACh. The action potential decreases in rising velocity and amplitude until it is completely depressed. Although the control response of this cell type is of low amplitude (see first frame), it is typical of recordings obtained from cells located in the middle AV ring region[6,7].

The embryonic heart cell, depending on its location, may be characterized by an action potential which consists of one or two components. It appears that nodal action potentials (e.g. N region of the embryonic AV ring[6]) consist only of the slow type response, which is ACh sensitive. On the contrary, transmembrane action potentials recorded from cells in the atrium and upper AV ring are typified by a fast (ACh insensitive) and slow (ACh sensitive) component. Similar findings have been reported for atrium, AN and N regions of the AV node of the adult rabbit heart, respectively[2]. These observations can be explained by assuming that the conductance changes responsible for myocardial depolarization (an initial rapid depolarization and a subsequent sustained depolarization) are processes which occur independently. They also suggest that the second process can appear in certain regions without an initial fast depolarization.

Some of the reported data differ from those of FINGL et al.[8] who have observed a decrease in the magnitude of both the resting and action potentials of the embryonic chick atrium and ventricle in the presence of acetylcholine. The present findings reveal a marked difference in response to ACh between atrium and ventricle, the latter being insensitive to the presence of the transmitter agent. Furthermore, the atrial resting membrane potential never decreases in the presence of acetylcholine. Thus, the present findings are in agreement with those reported for the adult mammalian heart[9].

With respect to the development of the heart, it is of interest to note that: (a) the earliest electrocardiographic records of the developing chick heart (33 h) are slow sinusoidal, rhythmic oscillations[10] comparable to the extracellular potentials recorded from the adult AV node[11] and (b) intracellular recordings both from early embryonic heart cells in vitro (37–72 h)[6,12] and pulsating vesicles cultivated from presumptive cardiac areas of blastoderm[13] are similar in configuration to those in the present study which consist of either 1-component (slow) or 2-component (fast-slow) action potentials. It is possible that a majority of the cells of the early heart (33 h) generate potentials which are characterized solely by the appearance of the slow component. With development, the ratio of 2-component to 1-component potentials may increase. Thus, at 38 h, when the embryonic ECG is characterized by sharp deflections (first manifestation of the QRS complex)[10], there may be present a preponderance of cells whose action potentials are characterized by a transient rapid component followed by a prolonged slow component. A definitive statement concerned with the electrical characteristics of the embryonic heart cells at very early stages of development awaits further experimentation[14].

Résumé. Le potentiel transmembranaire des cellules cardiaques de l'embryon de poulet est étudié ici par la technique des microelectrodes, pendant l'application localisée d'acétylcholine éjectée d'une micropipette. Le potentiel transmembranaire offre 2 processus différents: une dépolarization transitoire initiale à haute vélocité et une dépolarization prolongée et lente qui lui suite. L'intensité et la durée de ces 2 processus varient selon le lieu qu'occupe la cellule dans le cœur.

M. LIEBERMAN and A. PAES DE CARVALHO

Instituto de Biofisica, Universidade Federal do Rio de Janeiro (Brazil), 27th January 1967.

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