Effect of some arrhythmogenic agents upon the acetylcholine content of the rabbit atria

There is evidence for the importance of acetylcholine as an essential cellular constituent of cardiac tissue affecting cationic exchange and rhythmicity (Burn, 1969), and recently the acetylcholine content of the heart after drug-induced changes has been measured (Malhotra & Pundlick, 1965; Sharma & Parmar, 1967). We now report the effect of three arrhythmogenic agents, aconitine, digitalis and barium, on the amount of acetylcholine in the isolated atria of the rabbit.

Thirty-eight albino rabbits of either sex, 1·3–2·4 kg, were stunned by a blow on the head, and the hearts removed. Atria were dissected from surrounding tissues and suspended at 29° ± 0·5° in a 40 ml bath containing oxygen saturated Ringer-Locke solution containing (g/litre): NaCl 9·0, KCl 0·42, $CaCl_2$ 0·24, $NaHCO_3$ 0·5 and dextrose 1. Contractions were recorded kymographically by a spring lever with minimal inertia. After the preparation was allowed to equilibrate for 60 min, 0·2 to 0·4 ml of freshly prepared solution of the arrhythmogenic agent in Ringer-Locke was added to make a final concentration in the bath of aconitine nitrate, 10 μg/ml, deslanoside 10 μg/ml, barium chloride 200 μg/ml. This resulted in the development of arrhythmia as seen kymographically, and, in a few instances confirmed electrocardiographically. When the arrhythmia had lasted for 5 min, atria were removed for estimation of their acetylcholine content (Anand, 1952). The extraction of acetylcholine was made in 10 ml of eserinized Ringer solution at pH 4 at 90° to 100° (Anand, 1952). The assay was with the frog rectus abdominis muscle. All the drugs caused a statistically significant increase in the acetylcholine content of the atria (Table 1).

Table 1. *Acetylcholine content (μg/g of fresh tissue) of the rabbit atria during drug-induced arrhythmia*

Drug	Dose μg/ml	Number of rabbits	Mean ± s.e.	Significance value
Control	—	12	1·29 ± 0·23	—
Aconitine nitrate	10	8	1·96 ± 0·18	$P < 0·05$
Deslanoside	10	8	8·66 ± 1·08	$P < 0·001$
Barium chloride	200	10	6·34 ± 1·05	$P < 0·001$

Acetylcholine causes a loss of potassium from the heart resulting in electrophysiological changes conducive to the development of arrhythmias; quinidine depresses this cationic efflux. On this basis Holland (1957) believes quinidine to act by interfering with the acetylcholine system in the heart. Other antiarrhythmic drugs like propranolol, diphenylhydantoin and pentobarbitone share with quinidine the ability to reduce the acetylcholine content of the heart (Khanna & Madan, 1968; Madan & Khanna, 1970). Also, diphenylhydantoin converts a digitalis-induced arrhythmia to sinus rhythm with a corresponding reversal of the digitalis-induced potassium efflux (Scherlag, Helfant & others, 1968). Our experiments are consistent with the view that acetylcholine may also be involved in the production of cardiac arrhythmias by drugs.

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Effect of apomorphine and pimozide on synthesis and turnover of labelled catecholamines in mouse brain

Labelled tyrosine has been used for the study of catecholamine synthesis and turnover in brain *in vivo* (Udenfriend & Zaltzman-Nirenberg, 1963; Gordon, Reid & others, 1966; Sedvall, Weise & Kopin, 1968; Nybäck & Sedvall, 1970). This method has the advantage that concentrations of endogenous amines in brain are left unchanged. We have now examined the effects of apomorphine and pimozide on accumulation and disappearance of catecholamines formed in mouse brain from [^{14}C]tyrosine.

Apomorphine stimulates dopamine receptors in rat brain (Ernst & Smelik, 1966; Ernst, 1967). Andén, Rubenson & others (1967) and Roos (1969) presented evidence that apomorphine decelerates dopamine turnover in rat brain, possibly by activating a negative feed-back mechanism from the stimulated receptors. In a recent study Persson & Waldeck (1970) obtained results indicating that apomorphine accelerates noradrenaline turnover in mouse brain.

Pimozide is a potent neuroleptic drug (Sterkmans, Brugmans & Gevers, 1968; Haase, Blankenburg-Zahn & others, 1969) and is more effective than chlorpromazine and haloperidol in antagonizing apomorphine-induced stereotyped behaviour (Janssen, Niemegeers & others, 1968). This indicates that the drug is a dopamine receptor blocker. Chlorpromazine and haloperidol accelerate synthesis and turnover of catecholamines in brain (Carlsson & Lindqvist, 1963; Corrodi, Fuxe & Hökfelt, 1967; Nybäck, Borzecki & Sedvall, 1968), effects which probably are due to an activation of the presynaptic neuron as a consequence of the receptor blockade.

After an intravenous injection of [^{14}C]tyrosine to mice, the contents in brain of labelled dopamine and noradrenaline increase during the first 30 min (Nybäck & others, 1968). Between 2 and 7 h after the precursor administration, the labelled amines disappear from brain at rates that appear to be exponential and are not altered by synthesis inhibition with α-methyltyrosine (Nybäck & Sedvall, 1970). Thus, the disappearance of labelled amines during the mentioned time interval will be determined predominantly by the turnover rates of the amines.

Saline or drugs were administered 2 h after the intravenous injection of [^{14}C]tyrosine (10 μCi/animal, 355 mCi/mmol). Groups of animals were killed 2 and 7 h after the precursor administration and the contents in brain of endogenous tyrosine and labelled tyrosine, dopamine and noradrenaline were measured (Nybäck & Sedvall, 1970). In a separate experiment the effect of apomorphine and pimozide on endogenous dopamine and noradrenaline concentrations in brain was measured spectrophotofluorimetrically (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958).

Apomorphine reduced the content of endogenous noradrenaline to about 70% of controls, whereas pimozide caused a reduction of the dopamine level in mouse brain (Table 1).

Apomorphine retarded whereas pimozide accelerated the rate of disappearance of [^{14}C]dopamine from brain in comparison with saline-treated animals (Table 2).