Br. J. Pharmac. (1970), **39**, 309–316.

The effect of age, species and adrenaline on the recovery of isolated atria from anoxia

R. G. PENN*

Department of Pharmacology, Charing Cross Hospital Medical School, 62–65 Chandos Place, London, W.C.2

Summary

1. The rate of recovery of the force of contraction of isolated atria from different species of mature animals after 1 hour of anoxia was recorded.

2. Atria from rabbits of different ages were also exposed to anoxia. Atria from rabbits, ranging in age from 8 days to 3 years, were essentially similar in their recovery from anoxia, which was significantly slower than that of atria taken from rabbit foetuses at the 25th day of gestation.

3. Atria from human foetuses at 12–16 weeks of gestation recovered from anoxia much faster and more completely than any other atria studied and it is postulated that this may be due, in part, to the concentration of endogenous catecholamines being low.

4. The addition of propranolol to the organ bath had no effect on the recovery of atria from anoxia but prevented the action of added adrenaline, which manifests itself in a slowing of the rate and incompleteness of the recovery.

Introduction

The mammalian heart is highly dependent on oxidative metabolism and is unable to maintain normal contractility during even brief periods of lowered oxygen availability. Penn (1965) showed that the recovery of contraction of isolated atria from acute anoxia was very dependent on the length of time for which they had been anoxic. It is possible that the recovery of the isolated atria from anoxia is also affected by the age and the species of the animal from which they came. According to Mott (1961), "... from approximately mid-gestation until some time in the early neonatal period, the younger the animal of any given species, the longer it survives total anoxia, whatever index of survival is taken as the end point. ..." The duration of survival of isolated organ systems (for example spinal reflexes, heart action) of animals also decreases steadily with increasing maturity and reaches a minimum at an age that depends on the species of animal (Enzmann & Pincus, 1934; Selle, 1941; Selle & Witten, 1941).

Penn (1965) has shown that the recovery of the amplitude of contraction of isolated rabbit atria after fixed periods of anoxia was decreased if adrenaline or noradrenaline was present during the anoxic phase. Furthermore, prior treatment of the rabbits with reserpine or the presence of iproniazid or sodium nitrite in the organ bath enhanced the rate of recovery.

* Present address: Sterling Winthrop Research and Development, Winthrop House, Surbiton, Surrey.

The work described in this paper was performed in an attempt to delineate some of the effects of age and species on the recovery of isolated atria from anoxia. To obtain more information on the role endogenous catecholamines play in this recovery, additional experiments involving adrenaline and propranolol were carried out.

Methods

The preparations used were the isolated atria of rabbits, rats, mice, hens, pigeons, guinea-pigs, cats and human foetuses. They were set up in Locke solution gassed with 97% oxygen and 3% carbon dioxide and maintained at 30° C. The constitution of the Locke solution was (mmol/litre): NaCl 153·9, KCl 5·63, $CaCl_2$ 2·1, $NaHCO_3$ 5·95, glucose 5·54. The pH was 6·8.

Isotonic recordings

With the exception of the human foetal atria (which were available only occasionally) the atria were set up, three of the same species at a time, in the organ bath. The spontaneous contractions were recorded by isotonic levers writing one above the other on a kymograph, the magnification being $\times 6$–$\times 7$. Initially, the atria were often somewhat irregular in rate and amplitude of contraction but within 1 h these had become steady and the experiment was then started.

The human foetal atria were taken from foetuses which had been removed by hysterotomy for termination of pregnancy, mainly on psychiatric or social grounds. The foetuses were usually 12–16 weeks old as estimated by the date of the mother's last menstrual period, their weight and the crown–rump length. Immediately after removal from the uterus, the foetus was placed in a container immersed in ice for transport to the laboratory. The atria were usually isolated within 2 h post-operatively, though good and regular contractions could be obtained with atria taken from foetuses removed from the uterus up to 5 h previously.

Isometric recordings

Experiments were also performed with atria taken from rabbit foetuses at the 25th day of gestation (full term: 31 days). These atria were too small for isotonic recording and they were mounted on a holder and attached to a silicon strain gauge which formed part of a Wheatstone bridge connected to a Devices pen recorder. The responses of these foetal rabbit atria were compared with those of the human foetal atria and atria from mature rabbits whose isometric spontaneous contractions were similarly recorded.

Experimental procedures for obtaining anoxia

Atria were subjected to anoxia by replacing the oxygenated Locke soluting with Locke solution which had been previously gassed with 97% nitrogen and 3% carbon dioxide. The atrial rate and force of contraction quickly declined and diastolic arrest occurred within a few minutes. During the anoxic phase the bath fluid was constantly gassed with the nitrogen mixture. It was replaced after one hour with oxygenated Locke solution, gassed with 97% oxygen and 3% carbon dioxide, and the amplitude of contraction was recorded over the next hour (recovery phase).

The drugs used in these experiments were (−)-adrenaline (hydrochloride) and (±) propranolol (hydrochloride).

A stock solution of adrenaline, 5·4 mmol/l., pH 4, was freshly made each day and kept refrigerated till immediately before use, when it was diluted as required.

A stock solution of propranolol, 3·38 mmol/l., was also freshly made up each day in saline and diluted as required. In experiments involving propranolol, the Locke solution was changed to Locke solution containing 3·38 μmol/l. of propranolol and 15 minutes were allowed for it to exert its effect before proceeding further. Locke solution containing propranolol was then used throughout the remainder of the experiment. At the end of the experiment adrenaline was added to the bath to confirm the persistence and effectiveness of the β-adrenoceptor blockade. Under these conditions adrenaline, 0·27 μmol/l., which in the absence of propranolol was sufficient to increase the amplitude of contraction by 30%, had no effect and indeed it was necessary to increase the concentration of adrenaline to 27–270 μmol/l. before measurable effects on contraction could be obtained.

Results

Effect of species difference

If isolated atria from different species are exposed to anoxia for 1 h and their spontaneous isotonic contractions are recorded, it is found that they do not all recover from the anoxia with the same speed or to the same degree.

The rates of recovery of the amplitude of contraction of the isolated atria of the different species used in the present work fall into three groups: that of the rabbit, guinea-pig, mouse, and cat ; that of the pigeon and hen, and that of the rat (Fig. 1). The recovery of the hen and pigeon atria is noticeably slower and less complete than that of the other atria.

Effect of age

Figure 2 shows the recovery from 1 h of anoxia of the spontaneous isotonic contractions of atria taken from rabbits of varying age and of atria taken from 12–16 week old human foetuses.

There seems to be no marked difference in the rate of recovery from anoxia with any of the atria taken from rabbits varying in age from 8 days to 3 years. The rate of recovery of the human foetal atria is obviously very different and there is a characteristic rapid increase in the amplitude of contraction in the initial part of the recovery phase to values greater than that before the anoxic phase. This recovery then declines to values similar to those for the rabbit atria by the end of 1 h of the recovery phase.

Figure 3 shows the recovery of atria that had been exposed to 1 h of anoxia while their spontaneous contractions were recorded isometrically. The atria compared were those of the human foetus, rabbit foetus and mature rabbit. The complete and almost immediate recovery of the human foetal atria is again obvious and is very different in its time course from that of the other atria. The rabbit foetal atria do, however, recover significantly faster than atria from the mature animal and, for example, at the 15th minute of the recovery phase, the foetal atria have regained 76% of the control value of the amplitude of contraction as compared with 52% for the atria of mature animals ($0{\cdot}05 > P > 0{\cdot}02$).

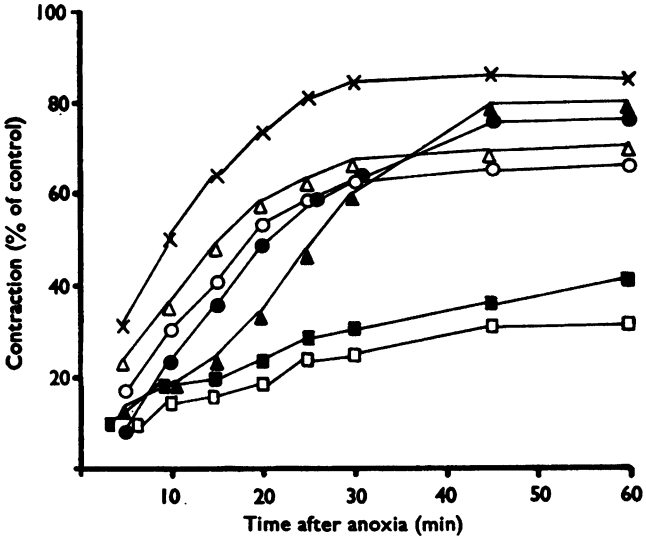

FIG. 1. Recovery of isolated atria from different species (means of six experiments for each species) from 1 h of anoxia at 30° C. Recovery is plotted as percentage of the amplitude before the anoxic phase. —●—, Rabbit; —○—, guinea-pig; —▲—, cat; —△—, mouse; —×—, rat; —■—, pigeon; —□—, hen.

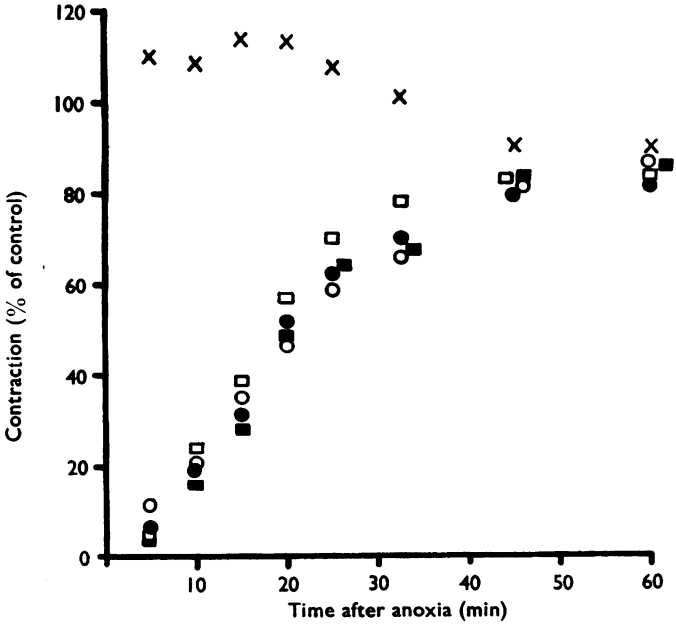

FIG. 2. Recovery of rabbit isolated atria from 1 h of anoxia at 30° C. Recovery is plotted as percentage of the amplitude of the spontaneous isotonic contractions before the anoxic phase. The atria were taken from rabbits of different ages and the recovery of atria taken from 12–16 week human foetuses is shown for comparison. ○, 8 day old rabbits (six atria); ●, 7 week old rabbits (four atria); □, 4 month old rabbits (four atria); ■, 3 year old rabbits (two atria); ×, human foetuses (three atria).

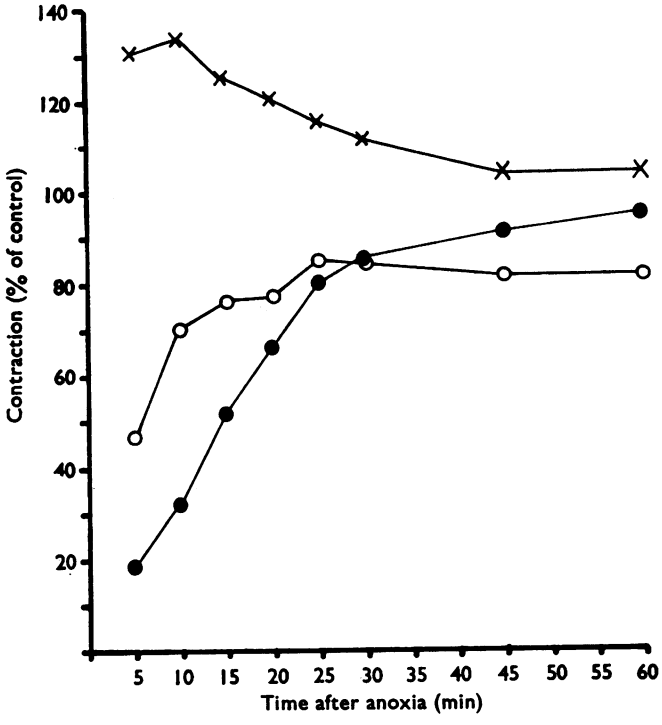

FIG. 3. Recovery of isolated atria from 1 h of anoxia at 30° C. Recovery is plotted as percentage of the amplitude of the spontaneous isometric contractions before the anoxic phase. Each curve represents the means of three experiments. —×—, Atria from human foetuses; —○—, atria from rabbit foetuses; —●—, atria from mature rabbits.

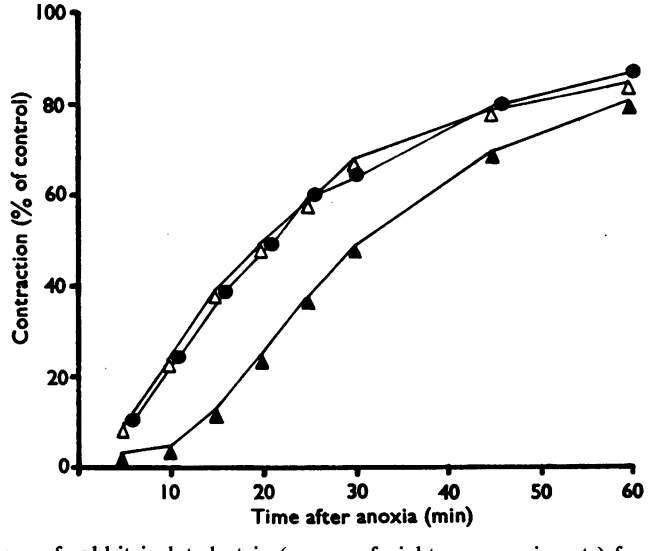

FIG. 4. Recovery of rabbit isolated atria (means of eighteen experiments) from 1 h of anoxia at 30° C when adrenaline (0·27 μmol/l.) was present during the anoxic phase. In some of the experiments the atria were also under the influence of propranolol (3·38 μmol/l.) throughout. Recovery is plotted as percentage of the amplitude of contraction before the anoxic phase. —●—, Control atria; —▲—, atria with adrenaline (0·27 μmol/l.) present during the anoxic phase; —△—, atria with adrenaline (0·27 μmol/l.) present during the anoxic phase and with propranolol (3·38 μmol/l.) present throughout.

Effect of β-adrenoceptor blockade

If rabbit atria are subjected to 1 h of anoxia when adrenaline (0·27 μmol/l.) is present during the anoxic phase, the recovery of the force of contraction after the anoxia is slower and less complete (Fig. 4). If the atria are, in addition, also under the influence of propranolol 3·38 μmol/l. (both during the anoxic and recovery phases) the slowing of recovery is not seen and recovery approximates that of the control atria.

Additional experiments showed that this concentration of propranolol has no effect on the recovery of isolated atria from 1 h of anoxia when adrenaline was not added during the anoxic phase.

Discussion

Effect of species

It might be thought that atria from the smaller animals with their higher metabolic rate (Lumb, 1963) would resist anoxia the least. However, atria from the rabbit, mouse, guinea-pig and cat seem to recover in an identical fashion although there was a thirty fold difference between the body weight of the heaviest donor animal (cat, 1,200 g) and that of the lightest donor animal (mouse, 40 g). The recovery of the force of contraction of atria from the pigeon and hen was slower and less complete than that of atria from the other animals and is thus in keeping with the idea that the rate of recovery from anoxia is at least in part dependent on metabolic rate. The rat atria recovered rapidly from anoxia compared with the other atria. That the rat often reacts in a different manner from other animals has been known for a long time and to quote Kruta and Braveny (1960) " . . . the contractility of the rat myocardium should not be considered representative of animals in general and one must be cautious of the interpretation of experimental results. . . ."

Effect of age

Rabbits of 2–3 months of age, weighing about 800 g, were used in a previous investigation of the recovery of isolated myocardium from anoxia (Penn, 1965). In the work described in this paper, it was found that atria from rabbits older than 8 days were essentially similar in their rate of recovery from anoxia.

Atria from rabbit foetuses do, however, recover significantly faster than atria from these older rabbits and it seems evident therefore that the resistance to anoxia of the rabbit atria reaches a steady level somewhere between the 25th day of gestation and the 8th postnatal day. This is in reasonable accord with the hypothesis discussed below that at least one factor influencing the recovery from anoxia is the amount of endogenous catecholamines.

The recovery of the human foetal atria is obviously different and is very rapid indeed and the amplitude of contraction in the first few minutes of the recovery phase is often greater than the control value. This rapid recovery is not solely due to the smallness of the human foetal atria, for atria from the rabbit foetuses were generally even smaller, yet had a much slower rate of recovery.

It is difficult to account for this behaviour of the human foetal atria and further experiments are contemplated to investigate it.

Effect of catecholamines

That endogenous catecholamines are concerned in the response of the isolated myocardium to anoxia was suggested by the work of Penna, Linares & Cáceres (1965) who found that, when isolated guinea-pig atria were made anoxic by bubbling nitrogen through the Tyrode solution bathing them, a significant increase in both amplitude and frequency of contraction occurred momentarily, followed by a decrease. Pretreatment of the donor animals with guanethidine or by chronic cardiac sympathectomy blocked this stimulant action and reserpine decreased it. They excluded any change in pH of the solution used as the cause of the stimulation and postulated that it was due to the liberation of endogenous catecholamines. Penna and his co-workers did not record the recovery from anoxia though they did find that chronic cardiac sympathectomy and pretreatment with bretylium gave (after an initial stimulant effect) a long lasting resistance to the effect of anoxia (personal communication).

This work is in keeping with the findings of Penn (1965), who showed that the recovery from anoxia was faster in atria whose catecholamines had been depleted by prior reserpinization of the donor animal. He also found that the recovery of isolated atria subjected to anoxia was slow and incomplete if adrenaline or noradrenaline were present during the anoxic phase.

The initial stimulation caused by the anoxic release of catecholamines is not therefore necessarily beneficial though it may be involved in the autoregulation of cardiac activity and coronary blood flow (Berne, 1963).

β-adrenoceptor blocking drugs such as propranolol are used in the treatment of angina pectoris. The rationale of this treatment is to protect the diseased myocardium from excessive sympathetic stimulation and consequent hypoxia. It has been shown by the results presented in this paper that β-adrenoceptor blocking drugs do not enhance the recovery of isolated atria from acute anoxia, in spite of being present in adequate blocking concentrations. If, however, during the period of anoxia the atria under the influence of propranolol are also exposed to adrenaline, the slow and incomplete recovery seen when adrenaline alone is present does not occur.

It seems probable therefore that propranolol will protect the myocardium from the effects of exogenous adrenaline present during the anoxic phase but not from that of endogenous adrenaline. This is in keeping with the findings of Shimamoto & Toda (1968) who found that propranolol antagonizes the β-adrenoceptor stimulating action of exogenous noradrenaline more effectively than the stimulating action of endogenous noradrenaline released by sympathetic nerve stimulation in isolated rabbit atria.

Recent work on the endogenous production of catecholamines during coronary occlusion in dogs is also interesting. Staszewska-Barczak & Ceremuzynski (1968) showed that, in the dog, the production of myocardial infarction by coronary occlusion led to a prolonged release of adrenaline by the adrenal medulla. The mechanism of this release is not known. Ceremuzynski, Staszewska-Barczak & Herbaczynska-Cedro (1969) showed that this output of adrenaline was correlated with the arrhythmias produced during the experimental infarction. In these circumstances the β-adrenoceptor blocking action of drugs such as propranolol would obviously be beneficial.

As reserpinization enhances the recovery of isolated atria from anoxia (Penn, 1965), it could be postulated that foetal atria are low in endogenous catecholamines and this may be part of the mechanism of their resistance to anoxia. There is no direct evidence on the comparative amount of catecholamines in the atria of the mature and foetal human heart, but Lee, McCarty, Zodrow & Shideman (1960) estimated the content of catecholamines in the hearts of chick embryos at varying stages of development. They found that the older embryos and the adult chicken have approximately 50% more catecholamines in their atria than the younger embryos. Friedman, Pool, Jacobowitz, Seagren & Braunwald (1968) measured the catecholamine content of the rabbit heart from the 29th day of gestation to 1 year of age. They found $0{\cdot}65 \pm 0{\cdot}065$ nmol/g of noradrenaline at term. This value rose rapidly sixfold by the end of the second week to $4{\cdot}08 \pm 0{\cdot}41$ nmol/g and then much more slowly to $6{\cdot}2 \pm 0{\cdot}89$ nmol/g by one year of age. These concentrations appear to be in keeping with the hypothesis discussed above and the experimental findings that isolated rabbit atria recover significantly faster from anoxia than atria from rabbits older than 8 days.

Work is in progress to assess the catecholamine content of the human foetal atria and on other possible mechanisms of its resistance to anoxia.

The Devices pen recorder was purchased by a joint grant from the University of London and the Wellcome Foundation.

REFERENCES

BERNE, R. M. (1963). Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. *Am. J. Physiol.*, **204**, 317–322.

CEREMUZYNSKI, L., STASZEWSKA-BARCZAK, J. & HERBACZYNSKA-CEDRO, K. (1969). Cardiac rhythm disturbances and the release of catecholamines after acute coronary occlusion in dogs. *Cardiovasc. Res.*, **3**, 190–197.

ENZMANN, E. V. & PINCUS, G. (1934). The extinction of reflexes in spinal mice of different ages as an indication of the decline of anaerobiosis. *J. gen. Physiol.*, **18**, 162–169.

FRIEDMAN, W. F., POOL, P. E., JACOBOWITZ, D., SEAGREN, S. C. & BRAUNWALD, E. (1968). Sympathetic innervation of the developing rabbit heart: biochemical and histochemical comparisons of fetal, neonatal and adult myocardium. *Circulation Res.*, **23**, 25–32.

KRUTA, V. & BRAVENY, M. (1960). Physiological differences between the myocardium of the rat and certain other animals. *J. Physiol., Paris*, **52**, 137–138.

LEE, W. C., MCCARTY, L. P., ZODROW, W. W. & SHIDEMAN, F. E. (1960). The cardiostimulant action of certain ganglionic stimulants on the embryonic chick heart. *J. Pharmac. exp. Ther.*, **130**, 30–36.

LUMB, W. V. (1963). *Small Animal Anaesthesia.* Philadelphia: Lea & Febiger.

MOTT, J. C. (1961). The ability of young animals to withstand total oxygen lack. *Br. med. Bull.*, **17**, 144–148.

PENN, R. G. (1965). Some factors influencing the recovery of isolated myocardium from acute anoxia. *Br. J. Pharmac. Chemother.*, **24**, 253–265.

PENNA, M., LINARES, F. & CÁCERES, L. (1965). Mechanism for cardiac stimulation during hypoxia. *Am. J. Physiol.*, **208**, 1237–1242.

SELLE, W. A. & WITTEN, T. A. (1941). Survival of respiratory (gasping) mechanism in young animals subjected to anoxia. *Proc. Soc. exp. Biol. Med.*, **47**, 495–497.

SELLE, W. A. (1941). Influence of age on survival of respiration, spinal reflexes, pupillary responses and heart action. *Proc. Soc. exp. Biol. Med.*, **48**, 417–419.

SHIMAMOTO, K. & TODA, N. (1968). Modifications by propranolol of the response of isolated rabbit atria to endogenous and exogenous noradrenaline. *Br. J. Pharmac. Chemother.*, **32**, 539–545.

STASZEWSKA-BARCZAK, J. & CEREMUZYNSKI, L. (1968). The continuous estimation of catecholamine release in the early stages of myocardial infarction in the dog. *Clin. Sci.*, **34**, 531–539.

(*Received February* 2, 1970)