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THE EFFECTS OF ANAESTHESIA INDUCED BY URETHANE OR PHENOBARBITONE UPON THE DISTRIBUTION OF PERIPHERAL CATECHOL AMINES IN THE RAT

BY

T. L. B. SPRIGGS*

*From the Department of Pharmacology, School of Pharmacy, Brunswick Square, London, W.C.*1

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In 1931, Sataké reported an increase in the plasma adrenaline level of dogs after an injection of urethane. Earlier, several workers had published indirect evidence that urethane released adrenaline from the adrenal medulla. For example, Aub, Bright & Forman (1922) detected an increase in the basal metabolic rate of intact but not of adrenalectomized cats during urethane anaesthesia; Seuffert & Ullrich (1925) and Conybeare, Densham, Maizels & Pembrey (1927) observed hyperglycaemia in animals treated with urethane, an effect which was abolished by cutting the splanchnic nerves (Kodama, 1930). More recently, Hökfelt & McLean (1950) detected a 40% depletion of adrenaline from the adrenal glands of a rabbit anaesthetized for 12 hr with urethane.

In the last decade the ability of adrenergically innervated tissues to take up exogenous or circulating catechol amines (Raab & Gigee, 1955) has been established. The possibility arose, therefore, that urethane anaesthesia affected the distribution of peripheral catechol amines. This was investigated and a comparison was made with the changes in catechol amine levels induced by phenobarbitone anaesthesia.

METHODS

Male Wistar rats, 150 to 250 g body weight, were given an anaesthetic dose, by intraperitoneal injection, of urethane (1.5 g/kg) or phenobarbitone sodium (100 mg/kg repeated at 8-hr intervals). Six rats from each group were killed at various times after injection. The heart, spleen and adrenal glands were removed, cleaned, weighed and stored at $-10°$ C until required for assay of catechol amine content.

Extraction and assay. Heart and spleen were extracted and assayed by the method of Shore & Olin (1958) as modified by Cass & Spriggs (1961). In most experiments the catechol amines were estimated in terms of noradrenaline, but in other experiments adrenaline and noradrenaline were estimated separately (Shore & Olin, 1958). All adrenal gland homogenates were analysed for both adrenaline and noradrenaline.

Infusions of adrenaline. Rats were anaesthetized with urethane (1.5 g/kg) and 80 min later given an infusion lasting 20 min of (−)-adrenaline (80 μg of base) into a femoral vein. The rats were killed 20 min after stopping the infusion.

* Present address: Department of Pharmacology, King's College, Strand, London, W.C.2.

Removal of adrenal medullae. Bilateral removal of the adrenal medullae was accomplished during ether anaesthesia by a method similar to that of Strömblad & Nickerson (1961). Control rats underwent a mock operation. All rats were left for 8 weeks before being used.

RESULTS

Adrenal glands. The adrenaline and noradrenaline contents of the adrenal glands at various times after induction of urethane anaesthesia are shown in Fig. 1,*a*. Fluctuations in the content of both amines were triphasic: an initial significant depletion was followed by a recovery towards the control value at 8 hr, after which a further significant depletion occurred. The percentage changes in the noradrenaline content were greater than those in the adrenaline content, although the alteration in the absolute amount of amine was greater for adrenaline.

The effect of phenobarbitone anaesthesia on the adrenaline and noradrenaline contents is shown in Fig. 1,*b*. No significant change was detected up to 4 hr but at 8 hr a significant depletion of both amines had occurred. The noradrenaline content increased to above control values at 16 and 24 hr, whereas the adrenaline content at these times was similar to the control values.

Heart. In intact rats the catechol amine content of the heart increased up to 8 hr after induction of anaesthesia with urethane, and was returning towards the control value at

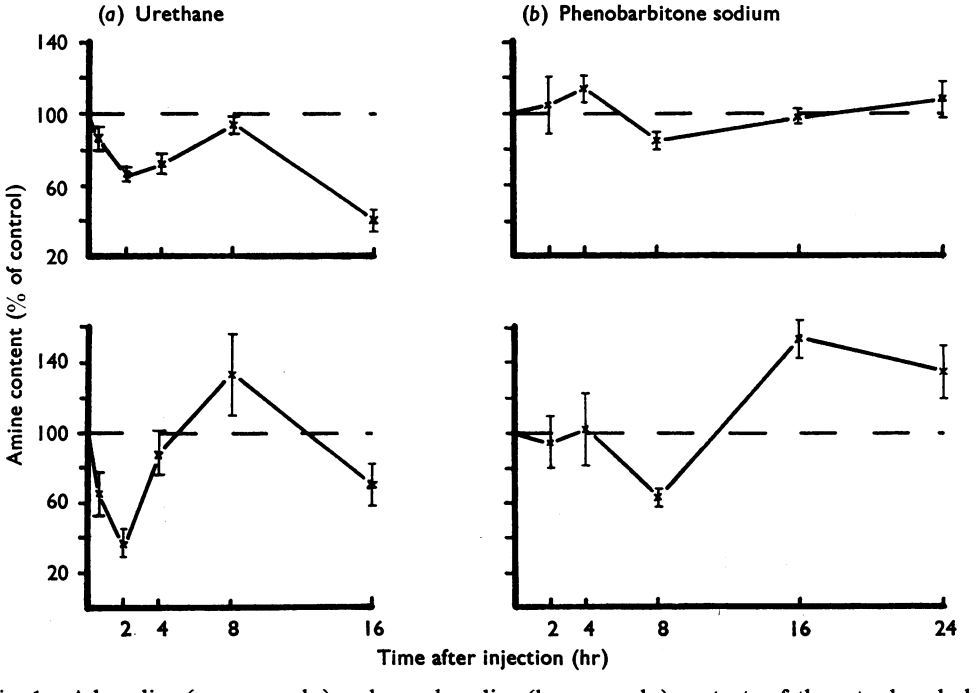

Fig. 1. Adrenaline (upper graphs) and noradrenaline (lower graphs) contents of the rat adrenal gland during anaesthesia induced by (*a*) urethane (1.5 g/kg) or (*b*) phenobarbitone sodium (100 mg/kg, at 0, 8 and 16 hr). The vertical lines represent the standard errors of the means of at least four pairs of adrenal glands.

16 hr (Fig. 2). In rats devoid of their adrenal medullae, however, urethane induced a pronounced decrease in the catechol amine content (Fig. 2).

The catechol amine content of the heart of intact rats was rapidly depleted during the initial 2 hr of phenobarbitone sodium (100 mg/kg) anaesthesia, although the level had returned to the control value at 8 hr (Fig. 3). Pentobarbitone sodium (60 mg/kg) anaes-

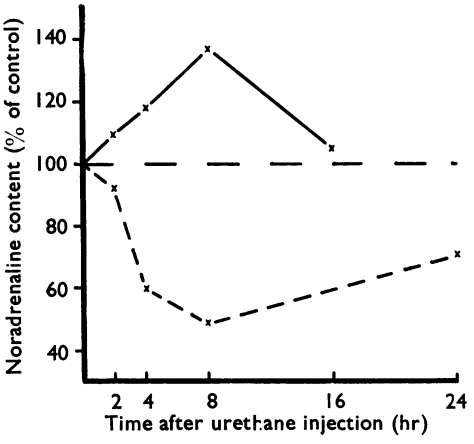

Fig. 2. The catechol amine content (estimated in terms of noradrenaline) of hearts from intact rats (×——×) and from those without adrenal medullae (×- - - -×), removed during urethane anaesthesia. Each point is the mean of at least three pools each of two hearts.

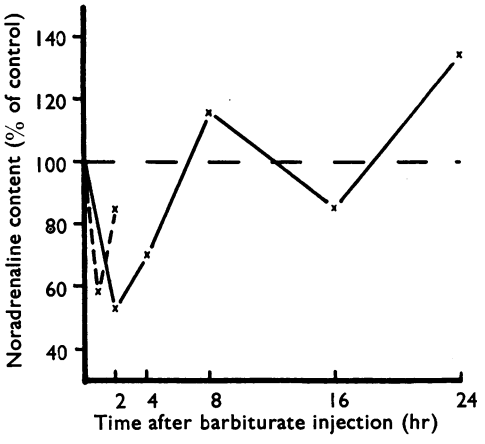

Fig. 3. The catechol amine content (estimated in terms of noradrenaline) of rat heart during barbiturate anaesthesia. ×- - - -×, Pentobarbitone sodium (60 mg/kg); ×——×, phenobarbitone sodium (100 mg/kg, at 0, 8 and 16 hr). Each point is the mean of at least three pools each of two hearts.

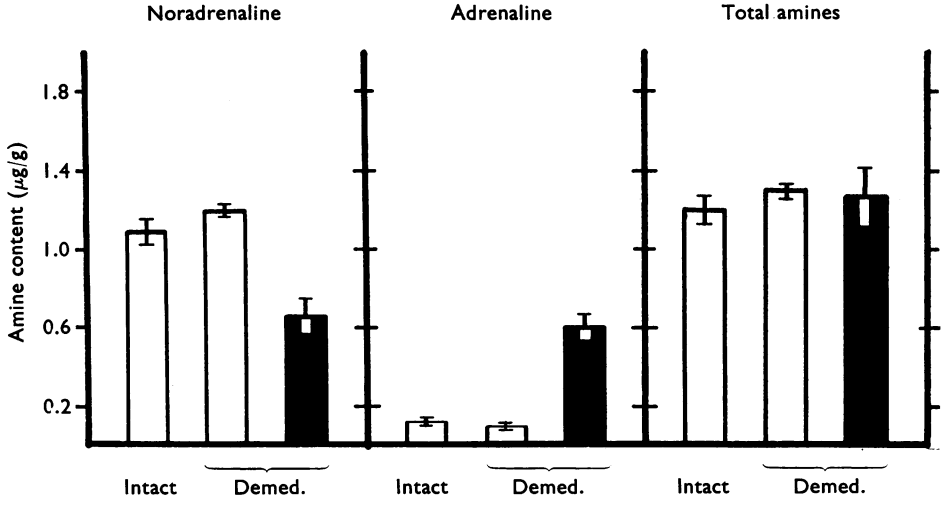

Fig. 4. The catechol amine content of hearts from intact rats and from those without adrenal medullae (Demed.) after infusion of adrenaline, during urethane anaesthesia. Empty columns, before infusion; filled columns, after infusion. The vertical lines represent the standard errors of the means.

thesia also induced a rapid loss of amines from the heart, but the recovery towards control values was more rapid (Fig. 3).

Adrenaline infusions. The rats in these experiments were killed exactly 2 hr after the administration of urethane, at which time little change had occurred in the catechol amine content of the heart (Fig. 2). After an infusion of adrenaline into rats with adrenal medullae removed, the cardiac content of adrenaline increased and that of noradrenaline decreased, although the total catechol amine content remained unaltered (Fig. 4). In the spleen, however, a small increase in adrenaline occurred which was not associated with a loss of noradrenaline (Fig. 5).

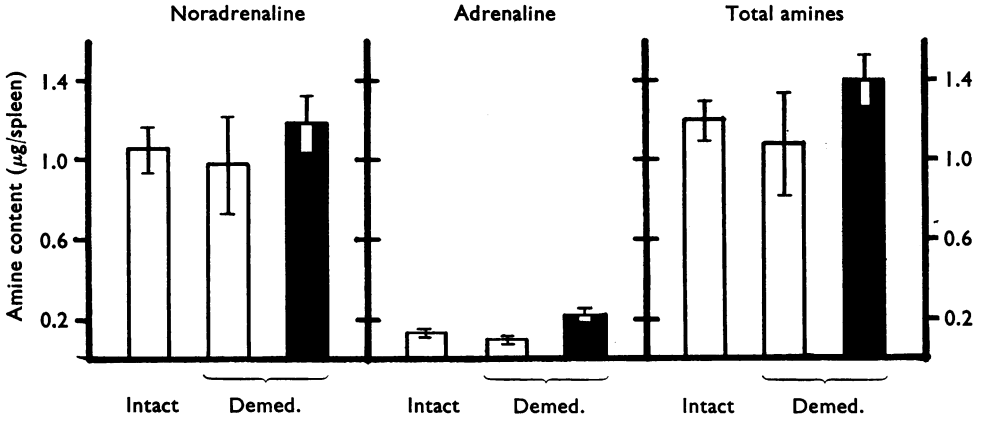

Fig. 5. The catechol amine content of spleens from intact rats and from those without adrenal medullae (Demed.) after infusion of adrenaline, during urethane anaesthesia. Empty columns, before infusion; filled columns, after infusion. The vertical lines represent the standard errors of the means.

Adrenaline constituted approximately 10% of the total catechol amines in the heart and spleen of intact rats, and this proportion was not changed in rats without adrenal medullae (Figs. 4 and 5).

DISCUSSION

In intact rats, urethane anaesthesia caused an initial depletion of adrenal catechol amines and a small increase in cardiac catechol amines. Although the adrenal content was restored at 8 hr it was again depleted at 16 hr. The changes in catechol amine content of heart and adrenal glands during the first 8 hr of phenobarbitone anaesthesia were the opposite of those occurring during the same period of urethane anaesthesia. It is concluded, therefore, that these changes were induced by the drugs and not by the condition of general anaesthesia *per se*. The changes occurring later than 8 hr after urethane may result from the long-term anaesthesia but interpretation of the results obtained later than 8 hr after phenobarbitone is speculative because the repeated injections of phenobarbitone complicate the picture.

Hyperglycaemia induced by urethane is abolished by transection of the brain caudal to the hypothalamus (De, 1946). In addition, Rogoff, Wasserman & Nixon (1946) have demonstrated the existence of a central inhibitory control mechanism which influences the

secretion of adrenaline from the adrenal medulla. Thus a lesion of the area bounded by the superior colliculi and the optic chiasma results in a threefold increase in adrenaline output from the adrenal gland. This region of the brain (the hypothalamus) is adjacent to the pituitary gland and is thought to modify the release of corticotrophin. It is interesting, therefore, that urethane anaesthesia induces a prolonged release of corticotrophin comparable with the increased adrenal medullary secretion, whereas phenobarbitone anaesthesia does not release either corticotrophin or adrenal catechol amines (Spriggs & Stockham, 1964). It thus appears probable that the alterations in peripheral catechol amine levels induced by the anaesthetics are mediated centrally.

Marley & Paton (1961) have calculated that about 0.2% of the adrenal medullary amines of the cat are released each minute during stimulation of the splanchnic nerves at a physiological frequency (Folkow, 1952) of 4 impulses/sec. This loss increased to 1 to 2%/min at a frequency of 20 impulses/sec. In the present experiments with urethane, 38.7% of the total adrenal amines were lost during the first 2 hr of anaesthesia, a rate of 0.32%/min. This rate of loss of catechol amines is therefore well within the physiological range which is expected if the release is mediated by way of the splanchnic nerves.

The catechol amine content of the heart of intact rats increased during the first 8 hr of urethane anaesthesia, during which time a considerable amount of amines was lost from the adrenal medulla. The possibility that this increase in heart content resulted from an uptake of circulating catechol amines is supported by the results of the experiments using rats deprived surgically of their adrenal medullae. The catechol amine content of the heart of these rats did not increase during urethane anaesthesia, but in fact decreased. When adrenaline was infused into the rats without adrenal medullae, a substantial increase in adrenaline content of the heart was found and this was associated with a comparable decrease in noradrenaline content. The total amine content of the heart was not changed. Other workers (Strömblad & Nickerson, 1961; Iversen, 1963) have observed an increase in adrenaline content over and above the decrease in noradrenaline content and it seems possible, therefore, that adrenaline displaces noradrenaline before occupying the additional empty storage sites in the tissue.

The small amount of adrenaline taken up by the spleen was not associated with a concomitant loss of noradrenaline and it appears that the uptake mechanism or the storage mechanism for catechol amines in the spleen differs from those in the heart.

In contrast to the increased adrenal medullary secretion in the presence of urethane, there is evidence that barbiturates depress the release of adrenal catechol amines (Hrubetz & Blackberg, 1938; Watts, 1951; Weil-Malherbe, 1955; Griswold & Mehlman, 1958). In the present experiments no change in adrenal catechol amine content was observed until 8 hr after giving phenobarbitone. Cardiac noradrenaline stores were depleted in intact rats anaesthetized with phenobarbitone and in rats with adrenal medullae removed and anaesthetized with urethane. It may be that cardiac noradrenaline is released in a metabolic capacity rather than in a neurohumoral transmitting role; that is, that these losses of noradrenaline are in response to the low plasma catechol amine levels. The noradrenaline content of the heart increased between 4 and 8 hr after phenobarbitone (Fig. 3) during which period a depletion of adrenal catechol amines occurred. This increase can be explained by the work of Bhagat & Shideman (1964), who have shown that noradrenaline liberated from the adrenal medulla may refill depleted cardiac stores.

It is generally accepted that volatile anaesthetics, such as ether and chloroform, stimulate the release of catechol amines from the adrenal medulla (Elliott, 1912; Elmes & Jefferson, 1942) and this has now been shown to be so for urethane. Urethane is a most useful anaesthetic in rabbits and rats and the effect of the drug on the peripheral catechol amine distribution may well modify experimental results—as indeed has been found by Bowman, Goldberg & Raper (1962).

SUMMARY

1. Anaesthetic doses of urethane or phenobarbitone sodium induced pronounced changes in the catechol amine contents of rat heart and adrenal glands.

2. The initial effects (up to 8 hr) appear to result from the action of the drugs themselves and not from the condition of general anaesthesia.

3. In intact rats urethane caused an increase in the cardiac content of catechol amines possibly resulting from an uptake by the heart of the amines released from the adrenal medulla.

4. Possible mechanisms by which the anaesthetics act to induce the observed changes in peripheral catechol amine levels are discussed.

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