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MODIFICATION OF CATECHOLAMINE CONTENT OF MALE ACCESSORY SEXUAL TISSUES OF GUINEA PIG AFTER PRETREATMENT WITH MICROSOMAL ENZYME INDUCERS

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Abstract—Recently, it was found that pretreatment of the guinea pig with phenobarbital increases the reactivity of the isolated seminal vesicle to indirect sympathomimetic agents. This was interpreted as either a direct effect on noradrenaline synthesis or an indirect effect via the acceleration of the biotransformation of endogenous steroids. To test these hypotheses, we studied the effect of phenobarbital pretreatment on the catecholamine content of various tissues of adult, castrated, and immature guinea pigs. The results show that phenobarbital pretreatment increases the noradrenaline content of the accessory sexual tissues, an effect that is not dependent on the hormonal state of the animals. It is suggested that microsomal enzyme inducers act locally to increase the synthesis of noradrenaline in terminal nerve endings. However, the possibility of an indirect action of phenobarbital via the adrenal gland remains to be verified.

TREATMENT of animals with many commonly used drugs, insecticides, and various other types of chemicals increases the level of liver microsomal enzymes that hydroxylate androgens, estrogens, corticoids, and progestational steroids.[1–4] This induction phenomenon is not restricted to the liver microsomes, however, and has been shown to occur in many other tissues.[5–8]

Recently, Gascon and Brodeur[9] reported that chronic treatment of guinea pigs with some microsomal enzyme inducers produces a marked increase in the reactivity of the isolated seminal vesicles to angiotensin or tyramine or to both. Since the stimulant action of these two compounds is mediated by release of catecholamines,[10,11] it was proposed that the inducers act either locally to stimulate the synthesis of noradrenaline or indirectly via the acceleration of the biotransformation of endogenous steroids in the liver.

In the present study, the influence of phenobarbital, 3-methylcholanthrene, and DDT on the catecholamine content of various tissues of the guinea pig was investigated. This effect was evaluated in mature, castrated, and immature guinea pigs.

MATERIALS AND METHODS

Intact adult (30-day-old), castrated adult, and immature (1-day-old) male guinea pigs were used in all our experiments. For each drug studied, the animals were divided into three groups and treated as follows: those of the first group were injected intraperitoneally with the microsomal enzyme inducers; the animals of the second group

received, simultaneously, the microsomal enzyme inducers and actinomycin D; and those of the last group served as controls and received appropriate vehicles only.

The animals were stunned and exsanguinated 24 hr after the last injection. The livers, adrenals, seminal vesicles, vas deferens, hearts, and brains were removed and homogenized in 5 vol. of cold perchloric acid (0·4 N). Homogenates were centrifuged at 9000 *g* at 0° for 10 min.

The 9000 *g* supernatant fraction was used for catecholamine determinations by the method described by von Euler and Lishajko.[12] Total protein estimations were performed by the method of Lowry *et al.*[13]

The following drugs were administered daily to the animals for 4 days: sodium phenobarbital, 50 mg/kg; 3-methylcholanthrene, 25 mg/kg; DDT, 50 mg/kg; actinomycin D, 7·5 μg/kg; and pargyline, 100 mg/kg.

The statistical analyses used were: determination of the standard error and the Student *t*-test.

RESULTS

Influence of phenobarbital on the noradrenaline and total protein content of various tissues of male adult guinea pigs. The noradrenaline content of the seminal vesicles, vas deferens, hearts, brains, livers and adrenals was measured after a 4-day pretreatment

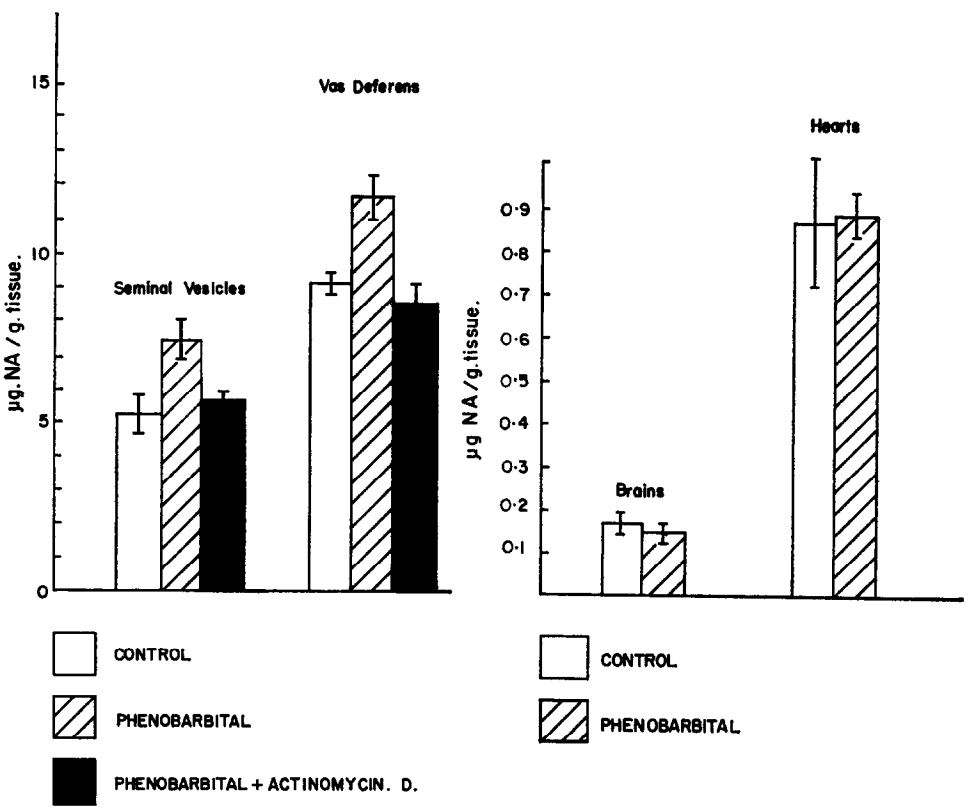

FIG. 1. Effect of pretreatment with phenobarbital (50 mg/kg/day for 4 days) and simultaneous pretreatment with actinomycin D (0·0075 mg/kg/day for 4 days) on the noradrenaline content of various tissues of the guinea pig. Vertical bars represent the standard error of the mean for 12 animals.

with phenobarbital (50 mg/kg/day). There was a significant increase ($P < 0.05$) in the noradrenaline content of the vas deferens and the seminal vesicles, while no apparent change in this parameter was observed in the brain and the heart (Fig. 1). On the other hand, the increase in noradrenaline content of the seminal vesicles and vas deferens was completely abolished by a simultaneous pretreatment with actinomycin D. In this connection, it is of interest to note that pretreatment with actinomycin D alone does not significantly modify the noradrenaline content of the seminal vesicles and vas deferens. The adrenaline content of the brain and heart remained unaltered and was practically nonexistent in the seminal vesicles and vas deferens.

As is seen in Fig. 2, pretreatment with phenobarbital did not alter the adrenaline and noradrenaline content of the liver; in contrast to this, the adrenals showed a decrease in noradrenaline and an increase in adrenaline after such pretreatment. The different treatments caused no significant modification in the total protein content of the seminal vesicle, vas deferens, and liver; protein determinations were not carried out on the adrenals. Furthermore, no significant difference in the weight of the seminal vesicles and vas deferens was observed between the control and phenobarbital-pretreated animals. The values for the seminal vesicle were $49{\cdot}20 \pm 3{\cdot}8$ mg and $57{\cdot}46 \pm 4{\cdot}6$ mg respectively; those of the vas deferens were $29{\cdot}41 \pm 1{\cdot}7$ mg and $31{\cdot}84 \pm 2{\cdot}1$ mg respectively.

Influence of 3-methylcholanthrene and DDT on the noradrenaline content of the seminal vesicles and vas deferens of adult guinea pigs. In this series of experiments, both 3-methylcholanthrene and DDT increased the noradrenaline content of the seminal vesicles ($P < 0.01$) and vas deferens ($P < 0.05$) after a 4-day pretreatment. Here also,

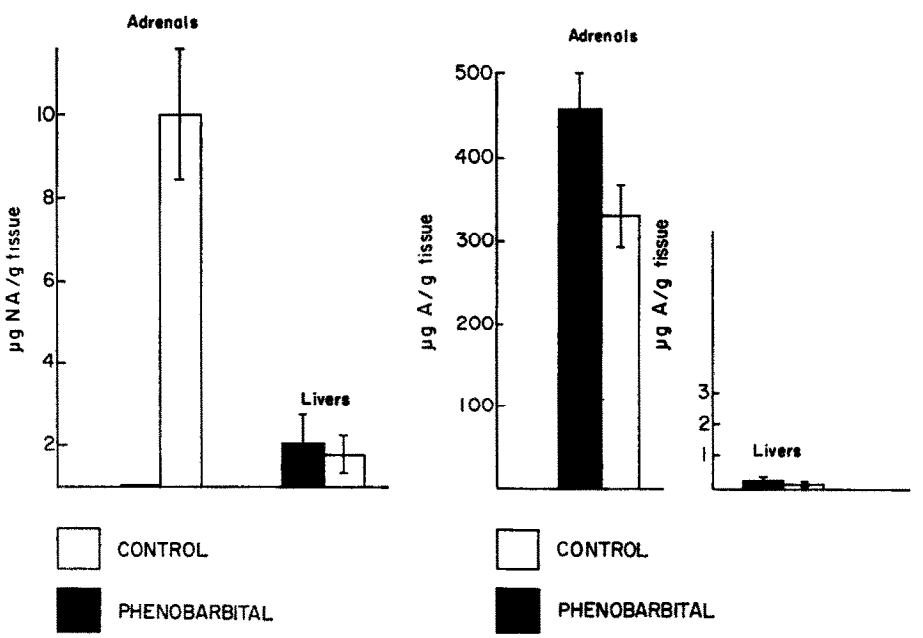

FIG. 2. Effect of pretreatment with phenobarbital (50 mg/kg/day for 4 days) on the noradrenaline and adrenaline content of the adrenals and liver of the guinea pig. Vertical bars represent the standard error of the mean for 12 animals.

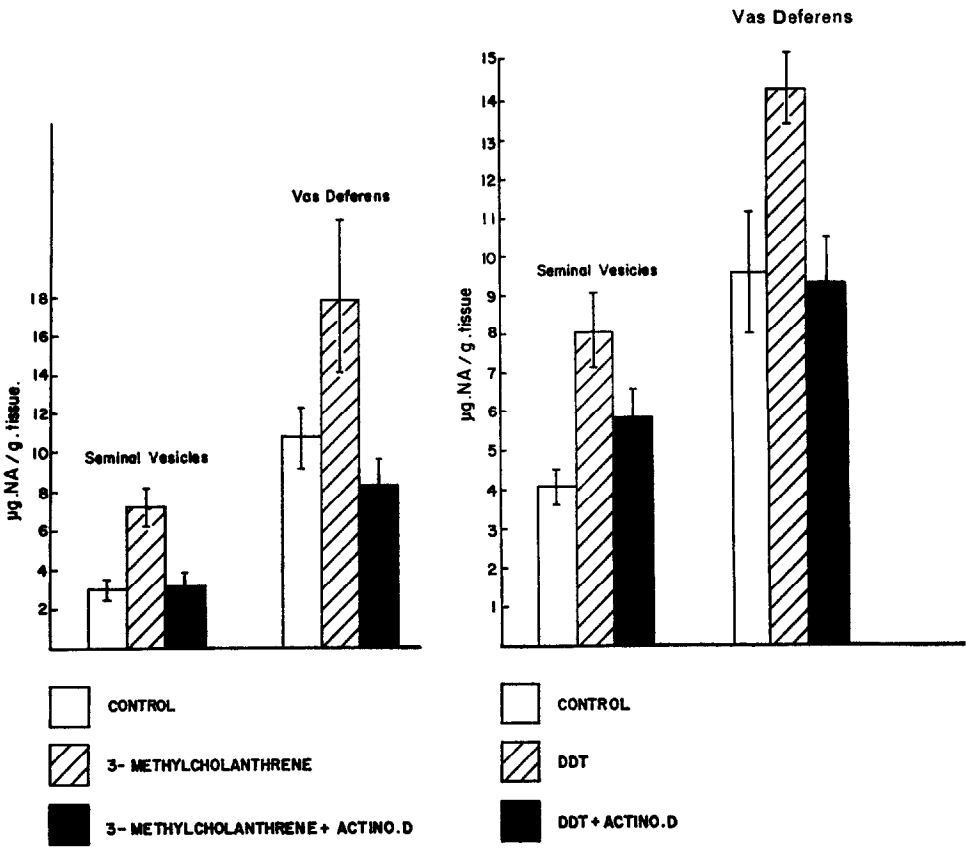

FIG. 3. Effect of pretreatment with 3-methylcholanthrene (25 mg/kg/day for 4 days), DDT (50 mg/kg/day for 4 days) and simultaneous pretreatment with actinomycin D (0·0075 mg/kg/day for 4 days) on the noradrenaline content of the seminal vesicle and the vas deferens of the guinea pig. Vertical bars represent the standard error of the mean for 12 animals.

the increase was abolished by actinomycin D (Fig. 3). No significant change was observed in the total protein concentration.

Influence of phenobarbital on the noradrenaline content of the seminal vesicles and vas deferens of adult castrated and immature guinea pigs. Castration of adult guinea pigs was performed 15 days after birth under light ether anesthesia. At the beginning of phenobarbital treatment, these animals were 45 days old and the immature animals were 1 day old. In the former group, pretreatment with phenobarbital still produced an increase in the noradrenaline content of the vas deferens ($P < 0{\cdot}05$) and seminal vesicle ($P < 0{\cdot}01$); in the latter group, the increase was practically 3-fold ($P < 0{\cdot}01$; Fig. 4). On the other hand, there was no apparent change in the amount of total proteins in any of the tissues studied.

Influence of pargyline on the noradrenaline content of the seminal vesicles and vas deferens of adult animals. In the last series of experiments, we studied the effect of pargyline, a monoamine oxidase (MAO) inhibitor, on the noradrenaline and total protein content of the vas deferens and seminal vesicles. The results in Fig. 5 show that pargyline increased the noradrenaline content of both in the vas deferens ($P < 0{\cdot}05$)

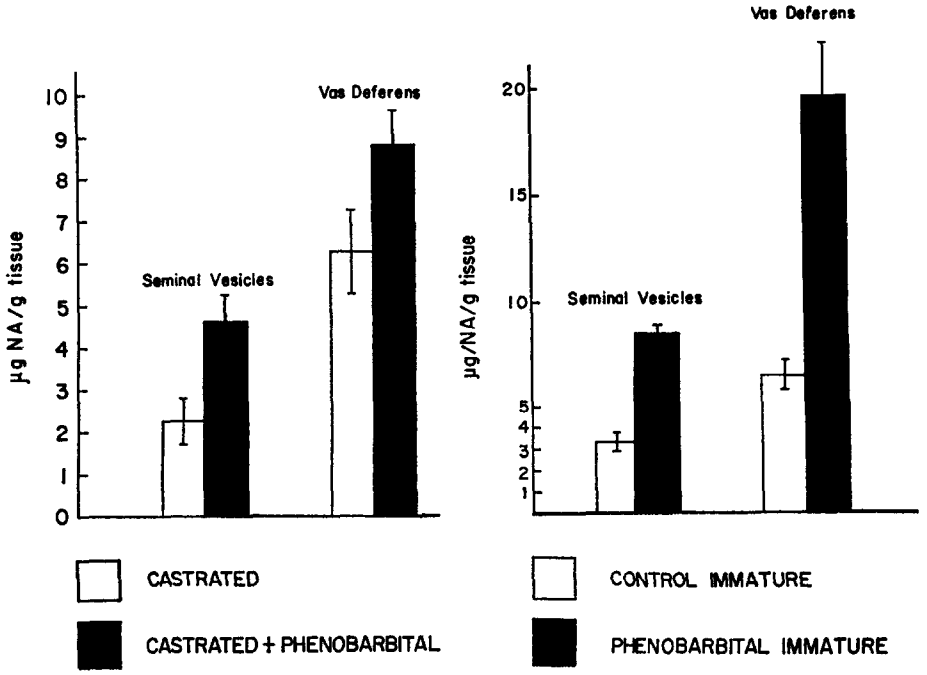

FIG. 4. Effect of pretreatment with phenobarbital (50 mg/kg/day for 4 days) on the noradrenaline content of the seminal vesicle and the vas deferens of the castrated adult and immature guinea pig. Vertical bars represent the standard error of the mean for 12 animals.

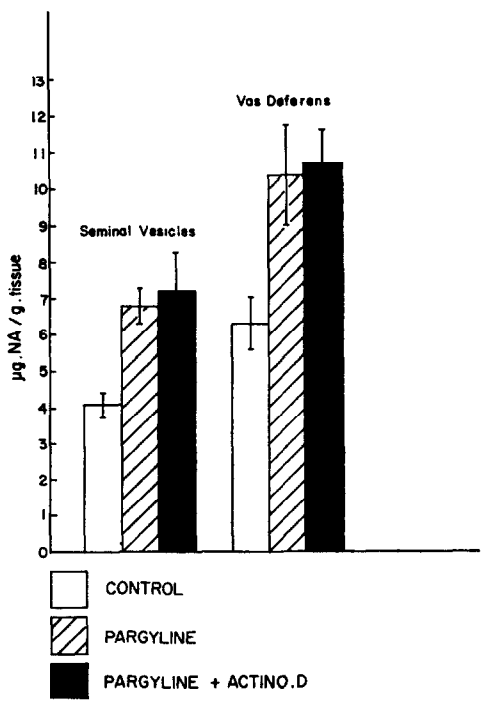

FIG. 5. Effect of pretreatment with pargyline (100 mg/kg/day for 4 days) on the noradrenaline content of the seminal vesicle and the vas deferens of the guinea pig. Vertical bars represent the standard error of the mean for 12 animals.

and seminal vesicle ($P < 0{\cdot}01$) without modifying the total protein concentration. Furthermore, this increase in noradrenaline was not altered by pretreatment with actinomycin D.

DISCUSSION

It was previously reported by Gascon and Brodeur[9] that pretreatment of guinea pigs with some microsomal enzyme inducers changes the reactivity of the isolated seminal vesicles to angiotensin and tyramine, two agents known to act via the liberation of endogenous catecholamines.[10,11] The results of the present work show that such pretreatment significantly increases the noradrenaline content of the seminal vesicles. Such was the case after subacute pretreatment of guinea pigs with phenobarbital, 3-methylcholanthrene, and DDT. A similar increase was also observed after pretreatment with pargyline, an MAO inhibitor. The fact that actinomycin D, an inhibitor of protein synthesis,[14] abolished the increase in noradrenaline content observed after pretreatment with the enzyme inducers but not after pargyline suggested an action of the former group of drugs via the synthesis of certain proteins.

To better localize the effect of the microsomal enzyme inducers, we studied the influence of phenobarbital pretreatment on the noradrenaline and adrenaline content of the vas deferens, adrenals, hearts, brains and livers. The results indicate that such pretreatment modifies the catecholamine content in the accessory sexual tissues and adrenals, only. In the adrenals, the increase in adrenaline content suggests an augmentation in the activity of the converting enzyme responsible for the transformation of noradrenaline into adrenaline. These last results indicate that the microsomal enzyme inducers act either locally or via increased hepatic biotransformation of endogenous steroids. If the second hypothesis is true, then pretreatment with phenobarbital should be less effective in castrated adults and immature animals, since, under these conditions, the androgenic hormones are practically absent. The findings reported here indicate that phenobarbital is as effective in castrated and immature guinea pigs as it is in the normal adult animals.

The hypothesis that phenobarbital acts directly on the accessory sexual tissues of the guinea pig to increase the noradrenaline content is supported by the observation of Côté *et al.*[15] These workers have reported that such pretreatment significantly increases the number of small dense-core vesicles in the adrenergic nerve endings of the seminal vesicles. However, our results do not eliminate completely an indirect action of phenobarbital via the adrenal gland. This possibility remains to be verified.

If we assume that phenobarbital acts directly, several mechanisms of action can be proposed. First, phenobarbital could increase the local synthesis of tyrosine hydroxylase. Since this enzyme is rate limiting in the synthesis of noradrenaline,[16] a local increase could result in increased noradrenaline. Second, phenobarbital could increase the axonal flow of small dense-core vesicles to the nerve endings, which in turn could increase the uptake of free noradrenaline. Since the amount of free noradrenaline seems to regulate the activity of tyrosine hydroxylase,[17] increased storage of noradrenaline in the small dense-core vesicles could result in a decrease in free noradrenaline, thus indirectly increasing tyrosine hydroxylase activity. Finally, phenobarbital could alter the permeability of the dense-core vesicles, facilitating the release of noradrenaline by indirect sympathomimetic agents. Unfortunately, the data do not permit us to differentiate among these various possibilities.

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