Br. J. Pharmac. Chemother. (1967), **31**, 26–31.

ENHANCEMENT OF NORADRENALINE PRESSOR RESPONSES IN TESTOSTERONE-TREATED CATS

BY

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(*Received February* 13, 1967)

During routine estimations of catecholamines in human urine by the spinal-cat blood pressure method, it appeared that male cats showed greater pressor responses to catecholamines than female cats. We therefore studied the effect of testosterone treatment on the pressor response to noradrenaline. Desmethylimipramine was also employed to obtain further enhancement of the pressor response.

METHODS

The results of the present study were obtained from 50 male and 19 female spinal cats weighing between 1.8–4.8 kg. Fifty-five cats were routinely anaesthetized by intraperitoneal injection of pentobarbitone sodium (30–35 mg/kg) and the spinal cord was then transected. In 14 cats the spinal transection was done under ether anaesthesia and the ether allowed to blow off. The femoral vein of one side was cannulated with an in-dwelling polyethylene cannula for administration of noradrenaline. Arterial blood pressure was recorded from the left common carotid artery by means of a mercury manometer and kymograph. All cats were bilaterally vagotomized and maintained on positive pressure artificial restiration. The spinal cord was exposed by laminectomy and two sutures were passed under the cord at the level of the second cervical vertebra. The cord was then transected between the sutures. After cord transection, the animals were allowed a rest period of 1 hr during which time the blood pressure became steady between 60 and 90 mm Hg.

In each experiment the minimal amount of noradrenaline required to elicit a detectable pressor response was determined. Subsequently, increasing doses of noradrenaline were injected every 4 min until a near maximal response was obtained. This provided one set of observations. In some cats when more than one set of observations was obtained, a period of 30 min rest was allowed between consecutive sets of observations.

Testosterone aqueous adsorbate (Aquaviron, Indian Schering) was administered to some cats intramuscularly in a single dose of 10 mg/kg, and these cats were used for the study on the second, third, fourth, fifth, sixth, seventh or ninth days after injection.

Desmethyl-imipramine (3 mg/kg) was administered to some cats intravenously 15 min before recording responses.

Solutions of noradrenaline were freshly prepared and kept in ice.

Analysis of data

The heights of the maximal pressor responses to noradrenaline in all the sets of observations in the control cats were measured and their mean calculated. This was the control average maximal

response. The mean response to a particular dose of noradrenaline obtained from the various sets of observations was expressed in terms of percentage of the control average maximal response. The mean values were then plotted against the corresponding log doses and the log dose percent response regression lines for noradrenaline were determined (Burn, Finney & Goodwin, 1950) in the control and drug-treated cats. From the regression lines it was possible to determine the amplitude of the pressor response to any given dose of noradrenaline in the control and the drug-treated groups.

The other criterion employed to assess the enhancement of noradrenaline responses by different treatments was the accurate determination of the average minimal dose required to elicit a detectable pressor response (height, 0.5 cm).

RESULTS

Noradrenaline pressor responses in the male and female cats under pentobarbitone sodium or ether anaesthesia

In a series of 30 spinal cats, variation in the noradrenaline pressor response was studied on the basis of sex and anaesthesia. Male cats were found to be about twice as sensitive as females to noradrenaline. There was no difference in the sensitivity of cats anaesthetized with ether or pentobarbitone. Table 1 shows the results. Under pentobarbitone anaesthesia, the average maximal pressor response in the group of 11 male cats was elicited with a dose of 12.3 μg (10.7–14.7) noradrenaline and the comparable response in the group of five female cats could be obtained with a dose of 29.0 μg (24.4–35.7). The greater sensitivity of the male cat to noradrenaline is further indicated by a comparison of the average minimal dose of noradrenaline required to elicit a detectable pressor response (see Table 1). Similarly, when the spinal cats were prepared under ether anaesthesia, the male cats were found to be more sensitive to noradrenaline than female cats. Cats given ether struggled a great deal, and since there was no significant difference between responses to noradrenaline under the two anaesthetics, sodium pentobarbitone was routinely used in subsequent studies. The sensitivity to noradrenaline in a second set of observations in the same cat was usually found to be greater than that observed in the first set of observations.

TABLE 1

NORADRENALINE (NA) RESPONSE IN MALE AND FEMALE CATS UNDER PENTOBARBITONE SODIUM OR ETHER ANAESTHESIA

Group of cats	Animals (No.)	Dose of NA (μg) to elicit average maximal response (95% fiducial limits)	Dose of NA (μg) to elicit average minimal detectable pressor response ($\pm$S.E.)
Male			
Pentobarbitone	11	12·3 (10·7–14·7)	0·17$\pm$0·029
Ether	3	11·9 (9·9–15·2)	
Female			
Pentobarbitone	5	29·0 (24·4–35·7)	0·4$\pm$0·038
Ether	11	27·8 (23·8–34·7)	

Effect of testosterone treatment in male cats

Since male cats were found to be more sensitive to the pressor effects of noradrenaline, the effect of exogenous testosterone on the noradrenaline pressor response was investigated. A single dose of 10 mg/kg testosterone aqueous adsorbate (Aquaviron) was given intramuscularly to 27 male cats. The results obtained from the testosterone-treated cats were compared with the untreated control group of 11 male cats.

TABLE 2

SENSITIVITY OF MALE SPINAL CATS TO NORADRENALINE (NA) ON VARIOUS DAYS FOLLOWING A SINGLE DOSE OF TESTOSTERONE (10 MG/KG)

Day following testosterone injection	Animals (No.)	Dose of NA (μg) to elicit average maximal response (95% fiducial limits)	Dose of NA (μg) to elicit average minimal detectable pressor response ($\pm$SE)	*P* value
Untreated (control)	11	12·3 (10·7–14·7)	0·17$\pm$0·029	
Second	3	105·3 (calculated)	0·51$\pm$0·22	>0·05
Third	5	17·7 (15·5–20)	0·2 $\pm$0·005	>0·05
Fourth	3	16·4 (14·6–18·5)	0·05$\pm$0·0008	0·01–0·05
Fifth	5	2·89 (2·77–3·02)	0·05$\pm$0·005	0·01–0·05
Sixth	4	2·67 (2·45–2·96)	0·02 $\pm$0·011	<0·001
Seventh	4	2·27 (1·90–3·25)	0·017$\pm$0·012	<0·001
Ninth	3	4·41 (3·96–5·01)	0·09 $\pm$0·010	>0·05

The cats were prepared for recording the carotid blood pressure on various days following testosterone injection. The results of this study are summarized in Table 2. A biphasic change in the responsiveness of the treated cats was observed: initially, there was a decrease in the sensitivity of the cats to noradrenaline, which was followed by a sharp increase in sensitivity. On the second day, maximal response obtained with 11 μg noradrenaline was only 54% of the control average maximal response, and the calculated dose noradrenaline required to elicit a response equal to the control average maximal response was 105.3 μg. Maximal enhancement of one noradrenaline pressor response was exhibited on the seventh day following testosterone injection, when the average maximal pressor response was elicited by a dose of 2.27 μg noradrenaline as compared with 12.3 μg for the untreated control. The minimal dose required to elicit a detectable pressor response on the eighth day was 0.017 μg compared with 0.17 μg for the control. The enhanced sensitivity began to diminish on the ninth day following testosterone injection.

Effect of combined treatment with testosterone and desmethylimipramine

Table 3 shows the results obtained from male cats treated with desmethylimipramine alone and in combination with testosterone. In 6 normal cats and 6 cats pretreated with testosterone seven days earlier, desmethylimipramine was injected intravenously 15 min before the set of observations with noradrenaline was obtained. The results are compared with a group of 11 untreated male cats which served as control. All cats were

made spinal on the day of testing. Desmethylimipramine enhanced the noradrenaline pressor responses and this enhancement was very pronounced in the testosterone-treated group. From this study it is clear that testosterone treatment enhanced the sensitizing effect of desmethylimipramine on the pressor response to noradrenaline.

TABLE 3

EFFECT OF COMBINED TREATMENT WITH DESMETHYLIMIPRAMINE ALONE AND IN COMBINATION WITH TESTOSTERONE ON THE ENHANCEMENT OF NORADRENALINE (NA) PRESSOR RESPONSES IN MALE SPINAL CATS

Group male spinal cats	Animals (No.)	Dose of NA (μg) to elicit average maximal response (95% fiducial limits)	Dose of NA (μg) to elicit average minimal detectable pressor response ($\pm$SE)	*P* value
Untreated	11	12·3 (10·7–14·7)	0·17–0·012	
Desmethylimpramine alone	6	7·35 (6·19–8·97)	0·07$\pm$0·024	<0·001
Desmethylimpramine +testosterone	6	0·900 (0·894–0·907)	0·011$\pm$0·004	<0·001

TABLE 4

EFFECT OF TESTOSTERONE TREATMENT (7TH DAY) IN SPINAL CATS

Sex of cats	Animals (No.)	Dose of NA (μg) to elicit average maximal response (95% fiducial limits)	Dose of NA (μg) to elicit average minimal detectable pressor response ($\pm$SE)	*P* value
Untreated				
Male	11	12·3 (10·7–14·7)	0·17 $\pm$0·013	
Female	5	29·0 (24·4–34·7)	0·4 $\pm$0·038	
Testosterone treated				
Male	4	2·27 (1·90–3·25)	0·017$\pm$0·012	<0·001
Female	3	3·43 (3·24–3·61)	0·009$\pm$0·003	<0·001

Effect of testosterone treatment in female cats

The results obtained in male cats on the seventh day following testosterone treatment as well as the results obtained from a group of 3 female cats injected with testosterone seven days earlier are shown in Table 4. The group of female cats exhibited a highly significant enhancement of noradrenaline responses following the testosterone treatment. The average maximal pressor response in the control female cats was obtained with a dose of 29 μg (24.2–35.7). This dose, after testosterone pretreatment, was reduced to 3.43 μg (3.24–3.61). A similar reduction was observable in the dose of noradrenaline required to elicit the minimal detectable response. It appears that testosterone treatment is more effective in enhancing the pressor response in female cats.

DISCUSSION

A decrease in the ED50 of a drug following treatment with another drug is commonly employed to assess potentiation. However, this method was found unsuitable in the present study because the ED50 of noradrenaline obtained from two sets of observations could be the same even though the amplitude of the pressor response to a given dose of noradrenaline differed significantly in the two sets. For the purpose of bioassay, enhancement of the amplitude of a response is more important than a decrease in the ED50. We therefore analysed the data as described above. As no significant difference was found in the dose of noradrenaline required to produce average maximal response in cats anaesthetized with pentobarbitone sodium or ether, pentobarbitone sodium was used in all subsequent experiments. It was easier to handle the animals under this anaesthetic.

Burn *et al.* (1950) said that male non-castrated cats are preferable to female cats for the bioassay of catecholamines. From the results of this study it is clear that male cats are about twice as sensitive to the pressor effect of noradrenaline as female cats.

The effect of exogenously administered testosterone was studied in male and female cats. Testosterone treatment markedly enhanced the pressor response to noradrenaline on the seventh day following a single injection of testosterone. The dose of noradrenaline required to elicit a detectable pressor response was one-tenth of the dose required in the untreated control cats. Similarly the dose of noradrenaline required to elicit a response equal to the control average maximal response was about one-sixth of the control dose (see Table 2). Such a magnitude of enhancement of the noradrenaline pressor response in cats has not been observed with several drugs known to enhance the catecholamine response. The enhancement was apparent after the fourth day and was maximal on the seventh day following testosterone injection. The enhancement was preceded by a period of relatively diminished sensitivity of the cats to noradrenaline. It is interesting that testosterone treatment enhanced the pressor response to noradrenaline even in female cats (see Table 3): testosterone treatment reduced the dose of noradrenaline required to elicit the maximal response to about one-ninth of the dose required in control females. Such an enhancement of noradrenaline responses with testosterone in male and female cats may be found valuable in the biological assay of the catecholamines. It is possible to enhance further the response to noradrenaline by combining testosterone treatment with desmethylimipramine treatment. The latter drug alone is capable of enhancing the noradrenaline pressor response but the enhancement is several times greater in testosterone-treated cats (see Table 4).

At present it is not possible to define the mechanism of the enhancement by testosterone. Enhancement could be the result of sensitization of the catecholamine receptors in some unknown manner. Alternatively, the protein anabolic action of testosterone might be responsible for providing additional catecholamine receptors. Yet again, the electrolyte retaining property of testosterone might be concerned in the enhanced pressor responses to noradrenaline, or testosterone could affect endogenous stores of catecholamines.

SUMMARY

1. Pressor responses to noradrenaline were determined in 69 spinally transected cats, bilaterally vagotomized and maintained on artificial respiration.

2. The cats were anaesthetized with ether or pentobarbitone sodium. The nature of the anaesthetic did not significantly affect the pressor response.

3. Male cats were found to be twice as sensitive to the pressor effects of noradrenaline as female cats.

4. A single injection of testosterone (10 mg/kg) markedly enhanced the noradrenaline pressor response on the seventh day. The enhancement was more apparent in female cats than in male cats.

5. Intravenous injection of desmethylimipramine enhanced the noradrenaline pressor responses and the enhancement was more pronounced in the testosterone-treated group.

6. This enhancement of the noradrenaline pressor response may improve the biological assay of catecholamines.

REFERENCE

BURN, J. H., FINNEY, D. J. & GOODWIN, L. G. (1950). *Biological Standardization*, pp. 26–176 and 219. 2nd ed. Oxford University Press, London.