Br. J. Pharmac. (1973), **46**, 667–675.

Effects of cocaine or denervation on responses of isolated strips of cat spleen to (-)-noradrenaline and (-)-isoprenaline

A. R. GRANATA AND S. Z. LANGER

Instituto de Investigaciones Farmacológicas, Consejo Nacional de Investigaciones Cientificas y Técnicas, Junín 956, 5° Piso, Buenos Aires, Argentina

Summary

1. A study has been made of the effects of cocaine and sympathetic denervation on responses of the cat spleen to (−)-noradrenaline and (−)-isoprenaline.

2. Responses of isolated strips of spleen capsule to (−)-noradrenaline or to (−)-isoprenaline were not affected by reserpine-pretreatment.

3. In adult cats, cocaine (1 and 10 μg/ml) or denervation produced a shift to the left of dose-response curves to (−)-noradrenaline, whereas they failed to modify dose-response curves to (−)-isoprenaline.

4. There was an increase in the maximum development of tension to (−)-noradrenaline after denervation or in the presence of cocaine (10 μg/ml). These procedures did not increase the maximal responses to (−)-isoprenaline.

5. Cocaine (10 μg/ml) did not affect dose-response curves to (−)-noradrenaline or (−)-isoprenaline in the denervated spleen.

6. In the kitten spleen, cocaine (1 and 10 μg/ml) produced a shift to the left of dose-response curves to (−)-noradrenaline both in untreated and in reserpine-pretreated tissues.

7. There was a small shift to the left in dose-response curves to (−)-isoprenaline in the presence of cocaine in the untreated but not in the reserpine-pretreated kitten spleen.

8. It is concluded that the potentiation of responses to (−)-noradrenaline in the cat's spleen is due to a prejunctional effect of cocaine.

Introduction

Potentiation of noradrenaline by cocaine or denervation is believed to be due to inhibition or abolition of neuronal uptake of amines (Trendelenburg, 1966; Langer, Draskóczy & Trendelenburg, 1967). A close correlation has been reported between the affinity of an amine for neuronal uptake sites and the degree of potentiation induced by cocaine or denervation (Trendelenburg, Muskus, Fleming & Gómez Alonso de la Sierra, 1962; Iversen, 1967; Trendelenburg, Maxwell & Pluchino, 1970). Yet, Davidson & Innes (1970) reported that cocaine potentiated both (−)-noradrenaline and (−)-isoprenaline in the cat's spleen, although cocaine blocked uptake of noradrenaline but not of isoprenaline in this tissue. These findings led the authors to conclude that inhibition of uptake is not the mechanism for the potentiation of noradrenaline obtained in the presence of cocaine. The aim of the present experiments has been to re-examine this problem by comparing the effects of denervation or cocaine on the responses of isolated strips of cat spleen to noradrenaline and isoprenaline.

Methods

Cats of 0·5 to 4·5 kg were anaesthetized with sodium pentobarbitone (35 mg/kg i.p.) and the trachea was cannulated. The spleens were removed through a midline abdominal incision and washed in Tyrode solution at room temperature and previously equilibrated with 95% O_2 and 5% CO_2. The composition of Tyrode solution was as follows (g/l): NaCl 8·0; KCl 0·20; $CaCl_2$ 0·20; $MgCl_2$ 0·01; NaH_2PO_4 0·05; $NaHCO_3$ 1·0; glucose 1·0; ascorbic acid 0·02 and sodium edetate 0·015.

Strips of spleen measuring approximately 20 by 7 mm were dissected and placed in an organ bath containing 10 ml of Tyrode solution which was bubbled with 95% O_2 and 5% CO_2 and maintained at 37° C. The upper end of the tissue was connected to a force displacement transducer (Grass FT 03) and the tension developed was recorded with a Grass Polygraph. A period of 40 min was allowed to elapse before starting the experiment. During this period, the Tyrode solution was replaced every 10 minutes. The resting tension of the muscle was repeatedly adjusted to 2·0 g and it reached a steady condition after 30–40 minutes. Dose-response curves to noradrenaline or isoprenaline were constructed by adding the drug cumulatively in such a way that the concentration in the bath was increased by a factor of about 3 whenever a steady response to the previous concentration had been reached. Only one dose-response curve was determined on each spleen strip.

When cocaine was used, it was added to the organ bath 20 min before the addition of noradrenaline or isolprenaline, and kept in the organ bath throughout the experiment. Pretreatment with reserpine (3 mg/kg, s.c.) was carried out 24 h before the experiment.

Denervation of the spleen: after premedication of the cat with 0·1 mg/kg of atropine sulphate subcutaneously, the left coeliac and superior mesenteric ganglia as well as the bulk of the coeliac plexus and part of the splenic plexus were removed under barbiturate anaesthesia.

All horizontal shifts of dose-response curves were measured at the level of the EC_{50}. For each dose-response curve the EC_{20} and EC_{80} were calculated. The slope of the dose-response curve was expressed as the quotient '60 divided by (log EC_{80} minus log EC_{20})' (Langer & Trendelenburg, 1969). Statistical calculations were performed according to conventional procedures (Snedecor & Cochran, 1969).

For tissue determinations of endogenous noradrenaline the spleens were blotted dry on filter paper, weighed and homogenized in cold 0·4 N perchloric acid containing 1 mg of sodium edetate and 1·25 mg of sodium sulphite per ml. The homogenates were kept at 4° C for 60 min and then centrifuged at 2000 rev/min for 10 minutes. An aliquot of the supernatant was brought to pH 8·2 with the addition of twice its volume of Tris buffer (0·5 M, pH 9). The aliquot was then poured into a column of 5 mm internal diameter packed with 200 mg of alumina. After passing the homogenate the column was washed with 5 ml of sodium acetate (0·2 M, pH 8·2) and 1 ml of water. Noradrenaline was eluted with 5 ml of 0·04 N perchloric acid. The fluorimetric determination of noradrenaline was carried out according to the method described by Laverty & Taylor (1968). The following drugs were used: cocaine hydrochloride; reserpine phosphate; (−)-noradrenaline bitartrate monohydrate, and (−)-isoprenaline hydrochloride.

Results

Effects of cocaine or denervation in adult cats

Since the spleen capsule of the cat lacks β-adrenoceptors (Bickerton, 1963) it was possible to elicit responses due to α-adrenoceptor activation with (−)-isoprenaline even in the absence of a β-receptor blocking agent. The concentrations of isoprenaline required to obtain responses were high (Fig 1B) and therefore

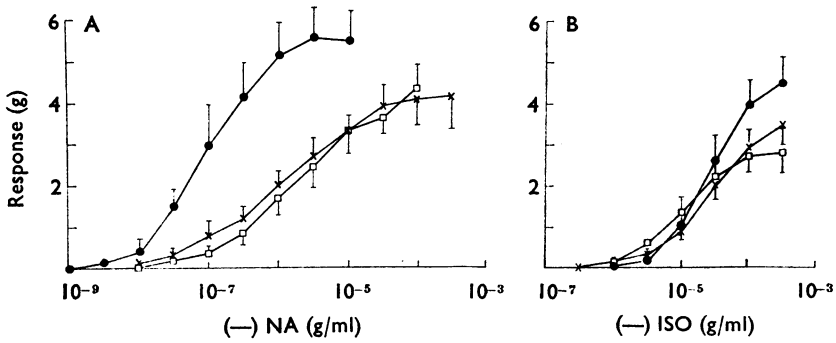

FIG. 1. Effects of reserpine-pretreatment and of surgical denervation on responses of isolated strips of spleen to noradrenaline and isoprenaline. A. Noradrenaline (NA); ×——× controls (n=19); □——□ reserpine pretreated (n=8); ●——● denervated, 7 days (n=7). B. Isoprenaline (ISO); ×——× controls (n=16) □——□ reserpine pretreated (n=7); ●——● denervated, 7 days (n=7). Shown are mean values ± S.E.M. n=number of experiments.

it was important to determine whether isoprenaline acts through the release of endogenous noradrenaline. Dose-response curves to noradrenaline or isoprenaline were unchanged in reserpine-pretreated spleens (Fig. 1, Tables 1 and 2), indicating

TABLE 1. *Effects of reserpine-pretreatment, cocaine and denervation on responses of isolated strips of cat spleen to (-) noradrenaline*

Groups	n	Log EC_{50} (ng/ml) (1)	Δ (2)	Maximal response (g) (3)	Slope (4)
(a) Control	19	3·224±0·121	—	4·16±0·43	43·0±2·1
(b) Reserpine-pretreated	8	3·269±0·100	−0·045(a-b)	4·44±0·47	39·9±1·9
(c) Cocaine 1μg/ml	5	2·188±0·209*	+1·036(a-c)	5·12±0·90	40·7±3·0
(d) Cocaine 10μg/ml Reserpine-pretreated	7	2·118±0·093*	+1·106(b-d)	5·84±0·48*	45·1±3·9
(e) Denervated 7 days	7	1·981±0·124*	+1·243(a-e)	5·78±0·43*	47·4±3·5
(f) Denervated plus cocaine 10 μg/ml	4	2·010±0·117*	−0·029(e-f)	5·73±0·87*	56·7±9·5

(1) The geometric means ±S.E. of the mean. (2) Δ Difference in log EC_{50} from the corresponding controls. Plus sign indicates shift to the left in the dose-response curve. (3) Maximum development of tension (grams). (4) Slope of dose-response curves: 60 divided by (log EC_{80} minus log EC_{20}). Results are given as mean values ±S.E. of the mean. n=number of experiments; *P<0·05 when tested against the control group.

that the effects of isoprenaline were of a direct nature. Seven days after surgical denervation there was a shift to the left in the dose-response curve to noradrenaline (Fig. 1A); the increase in sensitivity to noradrenaline was 16-fold (Table 1). Yet, there was no change in sensitivity to isoprenaline after denervation (Fig. 1B, Table 2).

TABLE 2. *Effects of reserpine-pretreatment, cocaine and denervation on responses of isolated strips of cat spleen to (–) isoprenaline*

Groups	*n*	Log EC_{50} (ng/ml) (1)	Δ (2)	Maximal response (g) (3)	Slope (4)
(a) Control	16	4·332±0·058	—	3·37±0·33	64·5±3·4
(b) Reserpine-pretreated	7	4·108±0·154	+0·224(a-b)	2·96±0·43	69·3±5·0
(c) Cocaine 1 μg/ml	5	4·123±0·116	+0·209(a-c)	2·21±0·33	41·4±4·3*
(d) Cocaine 10 μg/ml Reserpine-pretreated	6	4·114±0·149	–0·006(b-d)	3·93±0·60	58·8±5·6
(e) Denervated 7 days	7	4·375±0·084	–0·043(a-e)	4·64±0·63	75·3±5·6
(f) Denervated plus cocaine 10 μg/ml	3	4·306±0·179	+0·069(e-f)	3·71±0·53	66·7±7·4

(1) The geometric means ±S.E. of the mean. (2) Δ Difference in log EC_{50} from the corresponding controls. Plus sign indicates shift to the left in the dose-response curve. (3) Maximum development of tension(grams). (4) Slopes of dose-response curves: 60 divided by (log EC_{80} minus log EC_{20}). Results are given as mean values ±S.E. of the mean. *n*=number of experiments. * $P<0.05$ when tested against the control group.

In the presence of cocaine (1 μg/ml) there was a shift to the left in the dose-response curve to noradrenaline (Fig. 2A) but the dose-response curve to isoprenaline remained unchanged. When a higher concentration of cocaine was tested (10 μg/ml) reserpine-pretreated cats were used to avoid the production of tone by this concentration of cocaine, since it has been shown that an increase in tone distorts determinations of supersensitivity (Langer, 1966). There was no further shift to the left in the dose-response curve to noradrenaline in the presence of 10 μg/ml of cocaine, although there was an increase in the maximum development of tension (Fig. 2A, Table 1). This concentration of cocaine failed to potentiate responses to isoprenaline (Fig. 2B, Table 2).

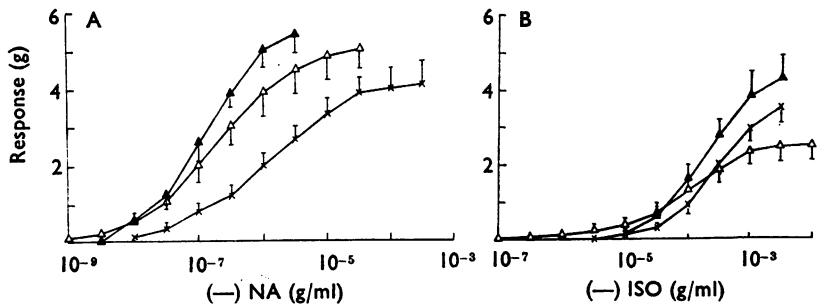

FIG. 2. Effects of cocaine on responses of isolated strips of spleen to noradrenaline and isoprenaline. A. Noradrenaline (NA); ×——× controls (*n*=19); △——△ cocaine 1 μg/ml (*n*=5); ▲——▲ cocaine 10 μg/ml in reserpine-pretreated tissues (*n*=7). B. Isoprenaline (ISO); ×——× controls (*n*=16); △——△ cocaine 1 μg/ml (*n*=5); ▲——▲ cocaine 10 μg/ml in reserpine-pretreated tissues (*n*=6). Shown are mean values±S.E.M. *n*=number of experiments.

The slopes of the dose-response curves for isoprenaline were steeper than those for noradrenaline in the normally innervated spleen (Tables 1 and 2; $P<0.001$).

There were no significant changes in the slopes of the dose-response curves for noradrenaline in the presence of cocaine or after denervation (Table 1). For isoprenaline there was a decrease in the slope of the dose-response curve in the presence of 1 μg/ml of cocaine (Table 2). However, there were no changes in the slopes of dose-response curves to isoprenaline for higher concentrations of cocaine or after denervation (Table 2). Consequently, the decrease

in slope observed in the presence of 1 μg/ml of cocaine might be related to the small development of tension reached (Table 2). The maximum development of tension in the control group was significantly higher for noradrenaline than for isoprenaline (Tables 1 and 2; $P<0{\cdot}05$). Neither cocaine nor denervation modified the maximum development of tension in dose-response curves to isoprenaline (Table 2). However, for noradrenaline, both cocaine (10 μg/ml) and denervation significantly increased the maximal responses (Table 1).

Effects of cocaine and denervation

The presynaptic component of denervation supersensitivity in the spleen is fully developed 4 days after the operation (Brimijoin, Pluchino & Trendelenburg, 1970). If the potentiation of noradrenaline or isoprenaline is due to postsynaptic

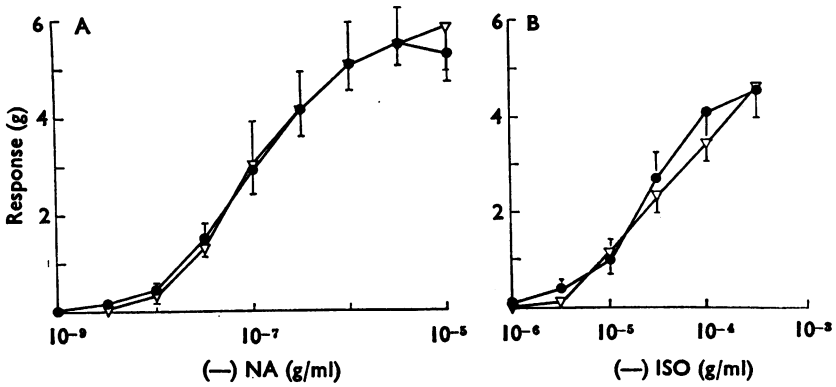

FIG. 3. Effects of cocaine on responses of the denervated spleen to noradrenaline and isoprenaline. A. Noradrenaline (NA); ●——● denervated, 7 days ($n=7$); ▽——▽ denervated 7 days, cocaine 10 μg/ml ($n=4$). B. Isoprenaline (ISO); ●——● denervated, 7 days ($n=7$); ▽——▽ denervated, 7 days, cocaine 10 μg/ml ($n=3$). Shown are mean values ± S.E.M. n=number of experiments.

effects of cocaine, these effects should be present in denervated tissues. Figure 3 and Tables 1 and 2 show clearly that cocaine did not potentiate noradrenaline or isoprenaline one week after denervation of the spleen.

Effects of cocaine in the kitten spleen

Davidson & Innes (1970) reported potentiation of isoprenaline by cocaine in cats of 0·6 to 1·2 kg. Our experiments, which did not show potentiation to isoprenaline, were carried out in adult cats, and therefore we considered the possibility that the difference in results was due to the age of the animals. Consequently, experiments similar to those described in the first section of **Results** were carried out in cats of 0·5 to 1·1 kg body weight. Table 3 shows the endogenous noradrenaline content of the spleen in kittens and in adult cats. The values for endogenous noradrenaline were significantly higher in the spleens of adult cats.

TABLE 3. *Endogenous noradrenaline content of spleen from adult cats and from kittens*

Groups	n	Body weight (kg)	Endogenous noradrenaline (μg/g)
Adult cats	15	2·34±0·1	2·90±0·29
Kittens	5	0·93±0·1**	1·39±0·20*

Results are given as mean values ±S.E. of the mean. *$P<0{\cdot}02$. ** $P<0{\cdot}001$.

Table 4 shows that the shift to the left in the dose-response curve to noradrenaline obtained in the presence of cocaine was nearly the same in the kitten spleen as in the adult spleen (Tables 1 and 4). As observed in the spleen of adult cats the shift to the left for noradrenaline was nearly maximal with 1 μg/ml of cocaine. The larger concentration of cocaine (10 μg/ml) did not elicit a further shift to the left in reserpine-pretreated tissues (Table 4); in control spleens,

TABLE 4. *Effects of reserpine-pretreatment and cocaine on responses of isolated strips of kitten spleen to (–) noradrenaline*

Groups	*n*	Log EC_{50} (ng/ml) (1)	Δ (2)	Maximal response (g) (3)	Slope (4)
(a) Control	10	3·389±0·122	—	4·41±0·48	37·3±1·6
(b) Reserpine-pretreated	5	3·464±0·113	–0·075(a-b)	4·04±0·38	31·0±1·3
(c) Cocaine 1μg/ml	6	2·048±0·102**	+1·341(a-c)	4·45±0·54	37·3±2·3
(d) Cocaine 10μg/ml	6	1·719±0·191**	+1·670(a-d)	4·90±0·69	—(5)
(e) Cocaine 10μg/ml Reserpine-pretreated	4	2·269±0·106**	+1·195(b-e)	3·77±0·59	42·9±0·8*

(1) The geometric means ±S.E. of the mean. (2) Δ Difference in log EC_{50} from the corresponding controls. Plus sign indicates shift to the left in the dose-response curve. (3) Maximum development of tension (grams). (4) Slopes of dose-response curves: 60 divided by (log EC_{80} minus log EC_{20}). (5) Values for slopes cannot be calculated in this group because of the presence of tone: 0·71±0·30g. Results are given as mean values ±S.E. of the mean. *n*=number of experiments. ** $P<0·001$. * $P<0·01$ when tested against the corresponding controls.

however, there was a further shift to the left which can be attributed to the development of tone rather than to inhibition of neuronal uptake of noradrenaline, since, in the cat's spleen, 1 μg/ml of cocaine elicits a nearly maximal inhibition of neuronal uptake of ^{3}H-noradrenaline (Cubeddu, Langer & Weiner, 1973.) For isoprenaline there was a small but significant shift to the left in the dose-response curve in the presence of 1 or 10 μg/ml of cocaine (Table 5). However, in the reserpine-pretreated kitten spleen cocaine failed to potentiate responses to iso-

TABLE 5. *Effects of reserpine-pretreatment and cocaine on responses of isolated strips of kitten spleen to (–)-isoprenaline*

Groups	*n*	Log EC_{50} (ng/ml) (1)	Δ (2)	Maximal response (g) (3)	Slope (4)
(a) Control	10	4·718±0·057	—	2·92±0·47	53·5±3·7
(b) Reserpine-pretreated	6	4·962±0·138	–0·244(a-b)	2·55±0·19	46·0±5·4
(c) Cocaine 1μg/ml	5	4·435±0·096*	+0·283(a-c)	4·06±0·70	48·5±3·1
(d) Cocaine 10μg/ml	6	4·254±0·086**	+0·484(a-d)	3·91±0·65	—(5)
(e) Cocaine 10μg/ml Reserpine-pretreated	6	4·875±0·163	+0·087(b-e)	3·36±0·36	49·4±3·4

(1) The geometric means ±S.E. of the mean. (2) Δ Difference in log EC_{50} from the corresponding controls. Plus sign indicates shift to the left in the dose-response curve. (3) Maximum development of tension (grams). (4) Slopes of dose-response curves: 60 divided by (log EC_{80} minus log EC_{20}). (5) Values for slopes cannot be calculated in this group because of the presence of tone: 0·58±0·19g. Results are given as mean values ±S.E. of the mean. *n*=number of experiments. * $P<0·02$. ** $P<0·001$ when tested against the corresponding controls.

prenaline although for noradrenaline the shift to the left was more than 1 log unit (Table 4 and 5). Since cocaine (10 μg/ml) elicited tone in untreated but not in reserpine pretreated spleens, potentiation of isoprenaline in the controls but not in reserpine pretreated tissues might be related to the presence of tone (Langer, 1966).

Discussion

In the cat's spleen the full development of the presynaptic component of denervation supersensitivity takes 4 days (Brimijoin *et al.*, 1970). Therefore, the presynaptic component of supersensitivity should be fully developed in the experiments performed one week after the operation. Green & Fleming (1968) reported the absence of a postsynaptic component in the supersensitivity developed after chronic denervation of the cat's spleen and consequently only the presynaptic component of denervation supersensitivity has been dealt with in our experiments.

In adult cats, cocaine or denervation caused a shift to the left in the dose-response curve to noradrenaline. Yet, there was no potentiation of isoprenaline in the presence of cocaine or after denervation. The maximum development of tension to noradrenaline was increased after denervation or in the presence of 10 μg/ml of cocaine, while for isoprenaline no such changes were observed. A similar increase in maximal responses was observed in the cat's nictitating membrane 3 days after denervation for noradrenaline but not for methoxamine (Langer, unpublished observations). Since noradrenaline has a high affinity for the neuronal uptake mechanism of amines, whereas methoxamine and isoprenaline do not (Iversen, 1967; Trendelenburg *et al.*, 1970) these results are compatible with the presynaptic origin of the increase in maximal responses after cocaine or short-term denervation. The failure of cocaine to inhibit uptake of isoprenaline in the cat's spleen (Davidson & Innes, 1970) indicates that this amine is taken up by extraneuronal structures rather than by adrenergic nerves (Iversen, 1967). Potentiation of noradrenaline but not of isoprenaline by cocaine or denervation in adult cats is therefore compatible with the presynaptic origin of both types of potentiation of noradrenaline.

Cocaine did not affect the sensitivity nor the maximal responses to either noradrenaline or isoprenaline in the denervated spleen. Similar results were obtained for noradrenaline in the denervated nictitating membrane of the cat (Langer *et al.*, 1967). However, Wakade & Krusz (1972) reported that cocaine induces a shift to the left in dose-response curves to noradrenaline in the denervated vas deferens of the guinea-pig. It appears that in some tissues cocaine, in addition to its presynaptic effect, also acts directly on the effector organ to increase its sensitivity to noradrenaline. Yet, these effects are usually of a small magnitude and while compatible with a postsynaptic effect should not be considered as evidence against the presynaptic component of action of cocaine.

The slopes of the dose-response curves for isoprenaline in the controls were steeper than those for noradrenaline. The latter was the more potent of the two amines. This difference follows the relationship between the potency of an amine and the slope of its dose-response curve described for sympathomimetic amines in the cat's nictitating membrane (Langer & Trendelenburg, 1969). However, we did not find a difference between the slopes of dose-response curves for noradrenaline between the normal and the denervated spleen.

In contrast to the results obtained in adult cats, in kittens there was a small shift to the left in dose-response curves to isoprenaline obtained in the presence of cocaine. Yet, after depletion of the noradrenaline stores by reserpine pretreatment, cocaine failed to potentiate responses to isoprenaline, although potentiation of noradrenaline remained unchanged under these experimental conditions. It is well known that cocaine retains its potentiating effects due to inhibition of neuronal uptake of noradrenaline after reserpine pretreatment (Trendelenburg,

1968 ; Langer & Trendelenburg, 1969). Furthermore, it is well known that 24 h reserpine pretreatment does not result in the postsynaptic type of supersensitivity that this drug elicits by chronic administration (Green & Fleming, 1968).

Consequently, it is likely that potentiation of isoprenaline in the presence of cocaine might be related to the development of tone in these tissues. A similar difference between the controls and reserpine-pretreated tissues in the shift of the dose-response curve to the left induced by cocaine was observed for noradrenaline: the shift was 0·5 log units larger in untreated spleens. The latter might again be due to the presence of tone since in our experiments the response was added to the underlying tone as recommended by Langer (1966). With the large concentration of cocaine (10 μg/ml) a small shift to the left was observed for isoprenaline in the cat's spleen by Trendelenburg, Hohn, Graefe & Pluchino (1971). Since these authors did not use reserpine-pretreated tissues it is uncertain whether their results can be attributed to the presence of tone and if the potentiation remains after reserpine-pretreatment.

Our results obtained in the kitten spleen differ from those of Davidson & Innes (1970) since after pretreatment with reserpine we found potentiation to noradrenaline but not to isoprenaline in the presence of cocaine. It is unlikely that this difference is due to the dose of reserpine employed (1 mg/kg by Davidson & Innes, and 3 mg/kg in our experiments), because both doses produce a maximal depletion of the noradrenaline stores (Langer, unpublished observations).

In addition, it was found that the endogenous noradrenaline levels were significantly higher in spleens of adult cats when compared to the tissues obtained from kittens. If the endogenous noradrenaline content is related to the density of adrenergic innervation (Trendelenburg, Draskóczy & Pluchino, 1969), it is reasonable to expect that if cocaine has a small postsynaptic effect it should be more evident in the tissue with the lower density of innervation. On the other hand, we cannot rule out a different interaction of isoprenaline and cocaine between the spleens from adult cats and from kittens because the difference in endogenous noradrenaline content between these groups is compatible with changes in the disposition of sympathomimetic amines. It should be stressed however, that most of the evidence which is discussed in the literature and which is relevant to the pre- or postsynaptic site of action of cocaine has been obtained in tissues from adult animals. Considering the differences described one should be cautious in extrapolating results obtained from young animals to adult ones.

Our results are compatible with a prejunctional effect of cocaine in the spleen of adult cats since neither cocaine nor denervation induced potentiation of isoprenaline while both procedures potentiated noradrenaline. In the kitten spleen the potentiating effect of cocaine is predominantly of presynaptic origin and the small potentiation of isoprenaline can be due either to the presence of tone, or to a very small postjunctional effect of the drug which could be observed in the normal but not in the reserpine-pretreated kitten spleen.

Our conclusions in connection with the prejunctional site of action of cocaine are similar to those reported recently by Trendelenburg, Graefe & Eckert (1972) in the cat's nictitating membrane.

The authors are indebted to Dr. S. Archer of Sterling-Winthrop Research Institute, U.S.A., for the supply of (−)-noradrenaline and (−)-isoprenaline.

REFERENCES

BICKERTON, R. K. (1963). The response of isolated strips of cat spleen to sympathomimetic drugs and their antagonists. *J. Pharmac. exp. Ther.*, **142**, 99–110.

BRIMIJOIN, S., PLUCHINO, S. & TRENDELENBURG, U. (1970). On the mechanism of supersensitivity to norepinephrine in the denervated cat spleen. *J. Pharmac. exp. Ther.*, **175**, 503–513.

CUBEDDU, L. X., LANGER, S. Z. & WEINER, N. (1973). The relationships between α-receptor block, inhibition of norepinephrine uptake and the release and metabolism of ^{3}H-norepinephrine. *J. Pharmac. exp. Ther.* (in press).

DAVIDSON, W. J. & INNES, I. R. (1970). Dissociation of potentiation of isoprenaline by cocaine from inhibition of uptake in cat spleen. *Br. J. Pharmac.*, **39**, 175–181.

GREEN, R. D. & FLEMING, W. W. (1968). Analysis of supersensitivity in the isolated spleen of the cat. *J. Pharmac. exp. Ther.*, **162**, 254–262.

IVERSEN, L. L. (1967). *The uptake and storage of noradrenaline in sympathetic nerves.* Cambridge University Press.

LANGER, S. Z. (1966). Presence of tone in the denervated and in the decentralized nictitating membrane of the spinal cat and its influence on determinations of supersensitivity. *J. Pharmac. exp. Ther.*, **154**, 14–34.

LANGER, S. Z., DRASKÓCZY, P. R. & TRENDELENBURG, U. (1967). Time course of the development of supersensitivity to various amines in the nictitating membrane of the pithed cat after denervation or decentralization. *J. Pharmac. exp. Ther.*, **157**, 255–273.

LANGER, S. Z. & TRENDELENBURG, U. (1969). The effect of a saturable uptake mechanism on the slopes of dose-response curves for sympathomimetic amines and on the shifts of dose-response curves produced by a competitive antagonist. *J. Pharmac. exp. Ther.*, **167**, 117–142.

LAVERTY, R. & TAYLOR, K. M. (1968). The fluorometric assay of catecholamines and related compounds: Improvements and extensions to the hydroxy-indole technique. *Anal. Biochem.*, **22**, 269–279.

SNEDECOR, G. W. & COCHRAN, W. G. (1969). *Statistical Methods.* Iowa State College Press, Ames.

TRENDELENBURG, U. (1966). Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **18**, 629–640.

TRENDELENBURG, U. (1968). The effect of cocaine on the pacemaker of isolated guinea-pig atria. *J. Pharmac. exp. Ther.*, **161**, 222–231.

TRENDELENBURG, U., DRASKÓCZY, P. R. & PLUCHINO, S. (1969). The density of adrenergic innervation of the cat's nictitating membrane as a factor influencing the sensitivity of the isolated preparation to l-norepinephrine. *J. Pharmac. exp. Ther.*, **166**, 14–25.

TRENDELENBURG, U., GRAEFE, K. H. & ECKERT, E. (1972). The prejunctional effect of cocaine on the isolated nictitating membrane of the cat. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **275**, 69–82.

TRENDELENBURG, U., HOHN, D., GRAEFE, K. H. & PLUCHINO, S. (1971). The influence of block of catechol-*O*-methyltransferase on the sensitivity of isolated organs to catecholamines. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **271**, 59–92.

TRENDELENBURG, U., MAXWELL, R. A. & PLUCHINO, S. (1970). Methoxamine as a tool to assess the importance of intraneuronal uptake of l-norepinephrine in the cat's nictitating membrane. *J. Pharmac. exp. Ther.*, **172**, 91–99.

TRENDELENBURG, U., MUSKUS, A., FLEMING, W. W. & GÓMEZ ALONSO DE LA SIERRA, B. (1962). Effect of cocaine, denervation and decentralization on the response of the nictitating membrane to various sympathomimetic amines. *J. Pharmac. exp. Ther.*, **138**, 181–193.

WAKADE, A. R. & KRUSZ, J. (1972). Effect of reserpine, phenoxybenzamine and cocaine on neuromuscular transmission in the vas deferens of the guinea pig. *J. Pharmac. exp. Ther.*, **181**, 310–317.

(*Received December* 29, 1972)