SHORT COMMUNICATION

INFLUENCE OF COCAINE, NIALAMIDE AND PYROGALLOL ALONE AND IN COMBINATION ON THE RESPONSES OF RAT ISOLATED SEMINAL VESICLE TO NORADRENALINE

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Summary: Lower doses of cocaine (0.75 and 3 *μg/ml*) produced only leftward shift of dose-response curve for the contractile effect of noradrenaline (NA) on the rat isolated seminal vesicle; higher dose (15 *μg/ml*) produced both leftward shift of the dose-response curve and increased the maximum response. Nialamide (3 *μg/ml*) or pyrogallol (1.3 *μg/ml*) or both did not appreciably alter cocaine-induced effects. It is concluded that cocaine-induced supersensitivity to NA is both prejunctional and postjunctional and that monoamine oxidase and catechol-O-methyltransferase inhibition does not enhance responses to NA.

Key words: rat isolated seminal vesicle cocaine nialamide pyrogallol prejunctional and postjunctional supersensitivity

INTRODUCTION

About 60% of infused noradrenaline (NA) is taken up by the adrenergic nerve terminals, 15% by the enzyme catechol-O-methyl transferase (COMT), 5% by the receptors and acceptors and about 20% overflows into the circulation (3). Some part of NA taken up into the adrenergic neurone is oxidatively deaminated by monoamine oxidase (MAO) which is localised in the mitochondria (14). Cocaine blocks the uptake of NA into the adrenergic nerve terminals (13, 18). Nialamide and pyrogallol which are MAO and COMT inhibitors respectively have no uptake blocking action (6, 8). The present investigation was, therefore, undertaken to determine the influence of cocaine alone and in the presence of pyrogallol and nialamide on the effects of NA on rat isolated seminal vesicle. The data thus obtained could provide information on the relative roles of different mechanisms on the disposition of NA.

MATERIALS AND METHODS

Seminal vesicles were obtained from male albino rats weighing 140-160 *g* and were prepared in the manner described by Leitch *et al.* (11). Preparations were suspended in a continuous flow of the bathing medium run at a rate of 10 *ml/min* and were attached to a balsa wood frontal writing lever placed under a tension of 300 *mg*. The lever gave 10-fold magnification. The ba-

thing solution was continuously bubbled with 5% carbon dioxide in oxygen. The temperature was maintained at 37°±0.5°C. NA was added at 5 min intervals. The flow was interrupted when NA was added to the bath. After obtaining control responses to increasing concentrations of NA (0.1-7 *μg/ml*), the preparations were equilibrated with fluid containing on concentration of cocaine and the dose-response curve was redetermined in the presence of cocaine. The cocaine containing bathing fluid was then replaced by the normal fluid and when the effect of cocaine had disappeared as determined by restoration of responses to at least 2 different concentrations of NA, nialamide was added to the bathing fluid. Fifteen min after equilibration with this solution responses to NA were redetermined in the presence of nialamide. The same procedure was repeated with pyrogallol, nailamide and cocaine, pyrogallol and cocaine and nialamide, pyrogallol and cocaine. Only one concentration of cocaine was studied in one experiment. At least 6-7 experiments were set up for each concentration of cocaine.

The mean response for each dose of NA was used for plotting the dose-response curves. The ED_{50} value was computed from the dose-response curve as the dose which corresponded to 50% its own maximum response. The ratio of control ED_{50} to that obtained in the presence of several procedures was used as a measure of the leftward shift of the dose-response curve.

Drugs: (—)-noradrenaline, (NA, Rhone Poulenc), cocaine hydrochloride (May and Baker), nialamide (Pfizer), pyrogallol (B.D.H.).

RESULTS

In the concentrations used nialamide or pyrogallol or cocaine had no effect of their own on the seminal vesicle. Nialamide (3 *μg/ml*) or pyrogallol (1.3 *μg/ml*) did not appreciably affect the dose-response curve for the contractile effect of NA. 0.75 *μg/ml* and 3 *μg/ml* of cocaine caused 1.7-fold and 2.6-fold leftward shifts respectively of NA dose-response curves without affecting the maximum responses. 15 *μg/ml* of cocaine produced a 3.2-fold leftward shift of NA dose-response curve and produced 125.7% increase in the maximum response. Nialamide (3 *μg/ml*) or pyrogallol (1.3 *μg/ml*)o f both decreased the leftward shift of NA dose-response curve produced by cocaine by 0.5 to 1.3 units. The increase in maximum response produced by cocaine was either slightly reduced or not affected by nailmide or pyrogallol or both. The data are summerised in Fig. 1 and Table I.

DISCUSSION

0.75 *μg/ml* and 3 *μg/ml* of cocaine produced 1.7 and 2.6-fold leftward shifts of the dose-response curve of NA respectively but did not increase the maximum response. 15 *μg/ml* of cocaine produced 3.2-fold leftward shift of dose response curve of NA and increased the maximum response by 125.7%. Cocaine by blocking the uptake of NA into adrenergic nerve endings (13, 18) would make larger amounts of NA available for action at the alpha-adrenergic receptor. This would be expected to result in leftward shift of the NA dose-response curve but no increase in maximum response. This component of cocaine induced supersensitivity would therefore be presyneptic.

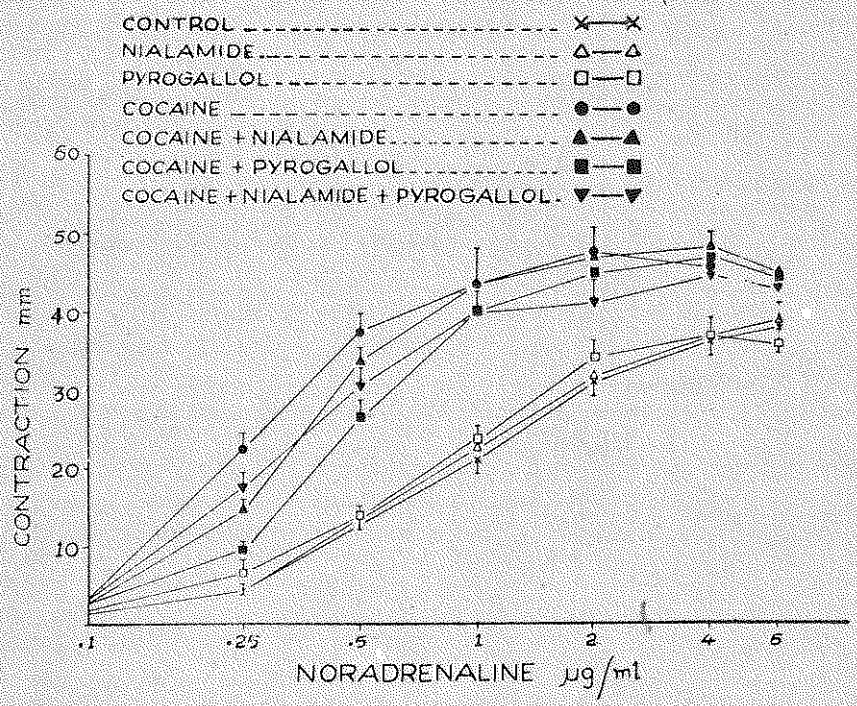

Fig. 1: Effect of cocaine (15 $\mu g/ml$), pyrogallol (1.3 $\mu g/ml$) and nialamide (3 $\mu g/ml$) alone and in combination (as shown n the key) on the dose-response curves for the contractile effect of noradrenaline on the rat isolated seminal vesicle preparations. Each point is the mean of 6-7 observations. Verticle lines indicate S.E.M.

TABLE I: Influence of cocaine, nialamide and pyrogallol on the ED_{50} values and maximal responses for the contractile effects of noradrenaline (NA) en rat isolated seminal vesicles.

Treatment		*ED_{50} of NA ($\mu g/ml$)*	*ED_{50} ratio**	*Maximum response (mm)*	*% of the control maximum response*
Control		0.85	—	37.8	100.0
Cocaine	0.75 $\mu g/ml$	0.49	1.7	38.5	101.8
	3 $\mu g/ml$	0.33	2.6	39.0	103.2
	15 $\mu g/ml$	0.26	3.2	47.5	125.7
Nialamide		0.80	1.06	38.8	102.6
Pyrogallol		0.70	1.20	38.0	100.5
Cocaine (0.75 $\mu g/ml$) + nialamide		0.65	1.30	38.2	101.0
Cocaine (0.75 $\mu g/ml$) + pyrogallol		0.69	1.20	38.3	101.1
Cocaine (3 $\mu g/ml$) + nialamide		0.40	2.1	37.4	98.9
Cocaine (3 $\mu g/ml$) + pyrogallol		0.60	1.4	40.2	107.1
Cocaine (3 $\mu g/ml$) + nialamide + pyrogallol		0.37	2.3	38.7	102.4
Cocaine (15 $\mu g/ml$) + nialamide		0.35	2.4	47.9	126.7
Cocalne (15 $\mu g/ml$) + pyrogallol		0.45	1.9	46.8	123.
Cocaine (15 $\mu g/ml$) + nialamide + pyrogallol		0.32	2.7	44.6	117.7

*Ratio of control ED_{50} to that obtained in the presence of different procedures.
Dose of nialamide was 3 $\mu g/ml$ and that of pyrogallol was 1.3 $\mu g/ml$

Our results on the increase in maximum response in addition to the leftward shift of the NA dose-response curve in the presence of cocaine are in agreement with those of Bevan and Verity (1), Kasuya and Goto (10) and Shah *et al.*, (15). Kasuya and Goto (10) reported that cocaine increased maximum response of the rat vas deferens to NA, angiotensin, ACh and K^+. They suggested that cocaine may be increasing maxmum responses to several agonists by facilitating the availability of Ca^{++} and concluded that this was a postsynaptic effect. A similar possibility could be suggested by our results. This component of cocaine induced supersensitivity would therefore be postsynaptic. In this respect our results are at variance with those of others who believe that cocaine-induced supersensitivity is predominantly postsynaptic (9,12). Others claim cocaine-induced supersensitivity to be largely presynaptic (5,17). In explaining the phenomenon of nonspecific desensitisation, Waud (16) introduced the concept of "Spare Cells". The results reported here on the increase in maximum responses to NA by cocaine would find support in this concept.

NA responses are potentiated by COMT inhibitors but not by MAO inhibitors (7). We did not observe appreciable leftward shift of NA dose-response curve with either inhibitor. However, the present results on the inability nialamide or pyrogallol to potentiate NA responses and the inability of either, or both to further increase cocaine-induced potentiation of NA responses are in accord with the reported failure of MAO or COMT inhibitors to increase the rate of urinary excretion of catecholomines (2,4). In fact, the cocaine-induced potentiation of NA responses was somewhat less when the preparations were subjected to simultaneous treament with nialamide or pyrogallol or nialmide and pyrogallol. No clearcut explanation can be offered for this unexpected result.

REFERENCES

1. Bevan, J. A. and M. A. Verity. Sympathetic nerve free vascular muscle. *J. Pharmac. Exp. Ther.*, **157** : 117-124, 1967.
2. Celander, O. and S. Mellander. Elimination of adrenaline and noradrenaline from the circulating blood. Nature, (London), **175** : 973-974, 1955.
3. Ferry, C. B. The autonomic nervous system. *Annu. Rev. Pharmac.*, **7** : 185-202, 1967.
4. Friend, G., M. S. Zileli, J. R. Hammelin and F. W. Reutter. The effect of ipronizid on the inactivation of norepinephrine in the human. *J. Clin. Exp. Psychopath.*, **19** : 61-68, 1958.
5. Green, R. D. and W. W. Fleming. Agonist and antagonist interactions in the normal and supersensitive nictitating membrane of the spinal cat. *J. Pharmac. Exp. Ther.*, **156** : 207-214, 1967.
6. Hertting, G., J. Axelrod and G. Whitby. Effect of drugs on the uptake and metabolism of H^3-norepinephrine. *J. Pharmac. Exp. Ther.*, **134** : 146-153, 1961.
7. Hattori, K. and S. Shibata. Effect of tranyleypromine on the mechanical activity of rabbit aorta and oesophagus and guinea pig taeniaacoli. *Eur. J. Pharmac.*, **5** : 291-295, 1969.
8. Iversen, L. L. The Uptake and Storage of Noradrenaline in Sympathetic Nerves. Cambridge University Press, London p. 155, 1967.
9. Kalsner, S. and M. Nickerson. Mechanism of cocaine potentiation of responses to amines. *Br. J. Pharmac.* **35** : 428-439, 1969.
10. Kasuya. Y. and K. Goto. The mechanism of supersensitivity to norepinephrine induced by cocaine in rat isolated vas deferens. *Eur. J. Pharmac.*, **4** : 355-362, 1968.
11. Leitch, J. L., C. S. Liebig and T. J. Haley. The use of rat's seminal vesicle for the assay of sympatholytic drugs. *Br. J. Pharmac.*, **9** : 236-239, 1954.
12. Maxwell, R. A., W. B. Wastilla and S. N. Eckhardt. Some factors determining the response of rabbit acrtic strips to dl-norepinephrine 7-H^3 hydrochloride and influence of cocaine, guanethidine and methylphenidate on these factors. *J. Pharmac. Exp. Ther.*, **151** : 253-261, 1966.

13. Muscholl, E. Effect of cocaine and related drugs on uptake of noradrenaline by heart and spleen. *Br. J. Pharmac.*, **16** : 352-359, 1961.

14. Sjoqvist, F. Toxic interactions between monoamine oxidase inhibitors (MAOI) and other drugs. In : Proceedings of the 3rd International Pharmacological Meeting, 1966 Sao Paulo, Edited by Cheymol, J. and J.R. Boissier. *Pergamon Press, London*, Vol. 10, p. **61**, 1968.

15. Shah, N. S., S. R. Patel and O. D. Gulati. Potentiation by 3, 4-dimethoxyphenylethylamine of responses to some drugs on guinea pig vas deferens. Under preparation. 1973.

16. Waud, D. R. Pharmacological receptors. *Pharmac. Rev.*, **20** : 49-88, 1968.

17. Westfall, D. P., D. C. McClure and W. W. Fleming. The effects of denervation decentralization and cocaine on the response of the smooth muscle of guinea pig vas deferens to various drugs. *J, Pharmac. Exp. Ther.*, **181** : 328-338, 1972.

18. Whitby, L. G., G. Hertting and J. Axelrod. Effect of cocaine on the dispposition of noradrenaline labelled with tritium. *Nature (Lond.)* **187** : 604-605, 1960.