Br. J. Pharmac. (1978), **63**, 651–657

COMPARISON OF THE RESPONSES OF SINGLE CORTICAL NEURONES TO TYRAMINE AND NORADRENALINE: EFFECTS OF DESIPRAMINE

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1 The technique of microelectrophoresis was used in order to compare the actions of tyramine and noradrenaline on single neurones in the cerebral cortex of the rat.

2 Tyramine could both excite and depress cortical neurones. Each tyramine-sensitive cell was also sensitive to noradrenaline. There was a high correlation between the directions of responses to tyramine and noradrenaline, most cells excited by tyramine being excited by noradrenaline, and most cells depressed by tyramine being depressed by noradrenaline.

3 In the case of both excitatory and depressant responses, tyramine appeared to be less potent than noradrenaline.

4 Tyramine evoked 'slower' responses than noradrenaline, both the latencies to onset and the recovery times being longer for responses to tyramine than for responses to noradrenaline.

5 When the rates of release of tyramine and noradrenaline from micropipettes were measured *in vitro*, no significant difference could be observed between the transport numbers of the two drugs. Thus the difference in potency between the two drugs, and the difference in the time courses of responses to the two drugs, are presumably of biological origin.

6 Desipramine could discriminate between neuronal responses to tyramine and noradrenaline: responses to tyramine were antagonized, while responses to noradrenaline were either potentiated or unaffected. Responses to DL-homocysteic acid were not affected by desipramine.

7 The results are consistent with the hypothesis that tyramine is an indirectly acting sympathomimetic amine in the brain, and desipramine acts by blocking the uptake of both tyramine and noradrenaline into presynaptic noradrenergic nerve terminals.

Introduction

Tyramine is generally believed to be an indirectly acting sympathomimetic amine, exerting its pharmacological actions by the release of noradrenaline (NA) from presynaptic stores (Burn & Rand, 1958; Trendelenburg, 1972). It has been reported that the tricyclic antidepressant drug, desipramine, blocks the uptake of tyramine into sympathetically innervated tissues (Brodie, Costa, Groppetti & Matsumoto, 1970) and thus antagonizes pharmacological responses to tyramine (Gessa, Vargin & Crabai, 1966; Fozard & Mwaluko, 1976). However, desipramine can also block the uptake of NA (Hertting, Axelrod & Whitby, 1961; Iversen, 1965) which may result in potentiation of the responses to NA (Sigg, Soffer & Gyermek, 1963; Sturman, 1970; McCulloch & Story, 1972). Thus desipramine and other uptake-blocking tricyclic antidepressants (e.g. nortriptyline), can discriminate between pharmacological responses to NA and tyramine: while responses to NA are potentiated, responses to tyramine are antagonized by the same concentration of the antidepressant (Barnett, Symchowicz & Taber, 1968; Fozard & Mwaluko, 1976; Doggrell & Woodruff, 1977).

It has been reported that desipramine can potentiate neuronal responses to NA in the brain (Hoffer, Siggins & Bloom, 1971; Bradshaw, Roberts & Szabadi, 1971; 1974; Bunney & Aghajanian, 1976). However, it is not known how neuronal responses to tyramine are affected by desipramine. In the experiments described here, we used the technique of microelectrophoresis in order to compare the agonistic activities of tyramine and NA on cortical neurones. We also examined whether desipramine can discriminate between neuronal responses to tyramine and NA.

Some of the results presented here have been communicated to the British Pharmacological Society (Bevan, Bradshaw, Pun, Slater & Szabadi, 1978a).

Methods

Pharmacological experiments

Male albino Wistar rats (250–350 g) were used in these experiments. The animals were anaesthetized with halothane (0.8 to 1.0%). Our methods for the surgical preparation of the animals, for the manufacture of six-barrelled micropipettes, for the extracellular recording of action potentials, and for the microelectrophoretic application of drugs have been described elsewhere (Bradshaw, Roberts & Szabadi, 1973a; Bradshaw, Szabadi & Roberts, 1973b; Bradshaw *et al.*, 1974; Bevan, Bradshaw & Szabadi, 1975a, b).

Six-barrelled micropipettes of tip diameter 3.0 to 5.0 μm were used. Two barrels of each micropipette contained 4.0 M NaCl, one for recording action potentials, the other for current balancing. The remaining barrels contained drug solutions. The following drug solutions were used: (−)-noradrenaline bitartrate (0.05 M, pH 3.0 to 3.5); tyramine hydrochloride (0.05 M, pH 5.0); DL-homocysteic acid (0.05 M, pH adjusted to 8.0 with NaOH); desipramine hydrochloride (0.005 M, pH 4.5). The pH of the drug solutions was adjusted only in the case of DL-homocysteic acid.

Spontaneously active neurones were studied in the cerebral cortex (stereotaxic coordinates, according to König & Klippel (1963): A 4.8–6.5, L 0.9–2.4). The area of recording was prepared as described previously (Bradshaw & Szabadi, 1972). The dura was either incised with a hypodermic needle, or was penetrated directly with the micropipette. All the drugs were applied by microelectrophoresis. When a suitable unit was encountered, the agonists were applied in a regular cycle. Between successive applications of agonists retaining currents of −10 nA were passed. Retaining currents of −25 nA were used for desipramine. Intervals between successive applications of the same agonists were kept constant in order to standardize the effects of the retaining current upon drug release during the ejection period (Bradshaw *et al.*, 1973a, b).

Equipotent current ratio was used as the measure of the relative potencies of tyramine and NA. The equipotent current ratio was defined as the ratio of ejecting currents (current for tyramine/current for NA) needed to evoke responses of approximately equal magnitude (maximum change in firing rate not differing by more than 20%) to the two amines.

Measurement of the release of tyramine and noradrenaline from micropipettes in vitro

Six-barrelled micropipettes were used in these experiments. Three barrels of each micropipette were filled with 0.05 M [sidechain 2-^{14}C]-tyramine hydrochloride (Radiochemical Centre, Amersham); the remaining three barrels contained 0.05 M [carbinol-^{14}C]-noradrenaline bitartrate (Radiochemical Centre, Amersham). The specific activities of both solutions were 1.0 mCi/mmole.

Our methods for the collection of samples have been described in detail elsewhere (Bradshaw *et al.*, 1973a). Sample collection periods of 10 min were used. Initially the rate of spontaneous release of radioactive material was measured in the absence of any electrophoretic current (4 samples). Then the rate of release of radioactive material was measured in the presence of ejecting currents of +25 nA, +50 nA, +75 nA and +100 nA (4 samples each) passed through each of the three barrels containing one of the amines. After a further 4 samples of spontaneously released radioactivity had been collected, the process was repeated for three barrels containing the other amine. The mean disintegrations per minute (d/min) obtained from the 8 samples of spontaneously released material were subtracted from the d/min obtained from each sample collected in the presence of an ejecting current; the remaining d/min were used for calculation of the rate of electrophoretic release. The transport number (n) of each amine was calculated from the following formula:

$$n = R_e zF/3i,$$

where R_e is the rate of electrophoretic release (mol/s), z is the valency (z = 1 for both tyramine and NA), F is Faraday's constant, and i is the intensity of the ejecting current (A) passed through each of the three barrels containing the amine in question.

Results

Comparison of agonistic effects of tyramine and noradrenaline

Responses of cortical neurones to tyramine Both excitatory and depressant responses to tyramine were observed in these experiments. Out of 165 cells responding to tyramine, 111 cells (67.3%) were excited, and 54 cells (32.7%) were depressed by the drug.

Comparison of directions of responses to tyramine and noradrenaline Each of the 165 cells responding to tyramine (see above), also responded to NA. On all but three cells the responses to tyramine and NA were in the same direction: of 111 cells excited by tyramine,

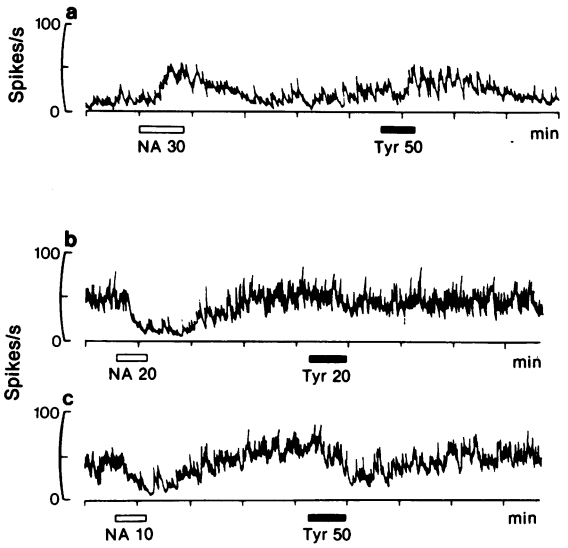

Figure 1 Comparison of apparent potencies of tyramine and noradrenaline. Ratemeter recording of the firing rate of two cortical neurones. Ordinates: firing rate (spikes/s); abscissae: running time (min). Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting current (nA). (a) Excitatory responses: in order to evoke approximately equivalent responses, a higher current was needed to apply tyramine (Tyr) than noradrenaline (NA). (b & c) Depressant responses: (b) Tyramine evoked a smaller response than noradrenaline when both drugs were applied with identical ejecting currents. (c) In order to evoke approximately equivalent responses, tyramine had to be applied with a higher current than noradrenaline.

110 were also excited by NA, and of 54 cells depressed by tyramine, 52 were also depressed by NA.

Comparison of apparent potencies of tyramine and noradrenaline The apparent potencies of tyramine and NA were compared on the same cells, by use of the equipotent current ratio (see Methods).

The relative potencies of the two drugs were compared on 67 cells (44 cells excited by both drugs, 23 cells depressed by both drugs). On each cell, in the case of both excitatory and depressant responses, tyramine appeared to be less potent than NA (see Figure 1): the mean equipotent current ratio (±s.e. mean) was 3.02 ± 0.22 for excitatory responses, and 2.52 ± 0.32 for depressant responses. In the case of both excitatory and depressant responses, the apparent potency of NA was significantly greater than that of tyramine (t test; $P < 0.001$).

Comparison of the time courses of responses to tyramine and noradrenaline Two time course parameters were measured: latency to onset and recovery time. Latency to onset was defined as the time elapsed between the beginning of the ejecting pulse and the onset of the neuronal responses; recovery time was defined as the time elapsed between the termination of the ejecting pulse and the recovery of the baseline firing rate (see Bradshaw *et al.*, 1973b). These parameters were measured on 67 cells giving approximately equivalent responses (in terms of the maximum changes in firing rate; see above) to the two amines (see Figure 2). Of the 67 cells, 44 cells were excited, and 23 cells were depressed by both drugs. The mean latencies and recovery times and the mean ratios of the time course parameters (tyramine/NA) are shown in Table 1. It is apparent from the table that the values of the ratios were significantly greater than one, indicating that tyramine evoked 'slower' responses than NA.

Effects of desipramine on neuronal responses to tyramine and noradrenaline

Desipramine, applied continuously from a dilute solution (0.005 M) with a low ejecting current (0 to 10 nA), usually did not have any effect on neuronal firing rate or spike amplitude. On some cells, however, a decrease in firing rate occurred, or the spike amplitude was reduced. Such cells were not used for drug-interaction studies.

The effects of desipramine upon responses to tyramine and NA were evaluated in the following way. When stable responses to the agonists had been

Table 1 Comparison of the time course parameters of neuronal responses to tyramine (Tyr) and noradrenaline (NA)

	Excitatory responses (n = 44)			*Depressant responses (n = 23)*		
Parameter†	*Value for Tyr*	*Value for NA*	*Ratio: Tyr/NA‡*	*Value for Tyr*	*Value for NA*	*Ratio: Tyr/NA*
Latency	25.61 ± 2.73	9.59 ± 1.60	4.84 ± 0.76**	23.70 ± 3.01	13.52 ± 1.31	2.49 ± 0.49*
Recovery time	173.05 ± 8.02	88.61 ± 6.68	2.25 ± 0.15**	139.91 ± 11.80	92.17 ± 6.19	1.60 ± 0.16**

† Time course parameters are in seconds.

‡ Ratios were calculated separately for each individual cell. Values are means ± s.e. mean for all cells. Significance levels (ratios significantly greater than 1; t test): * $P < 0.01$; ** $P < 0.001$.

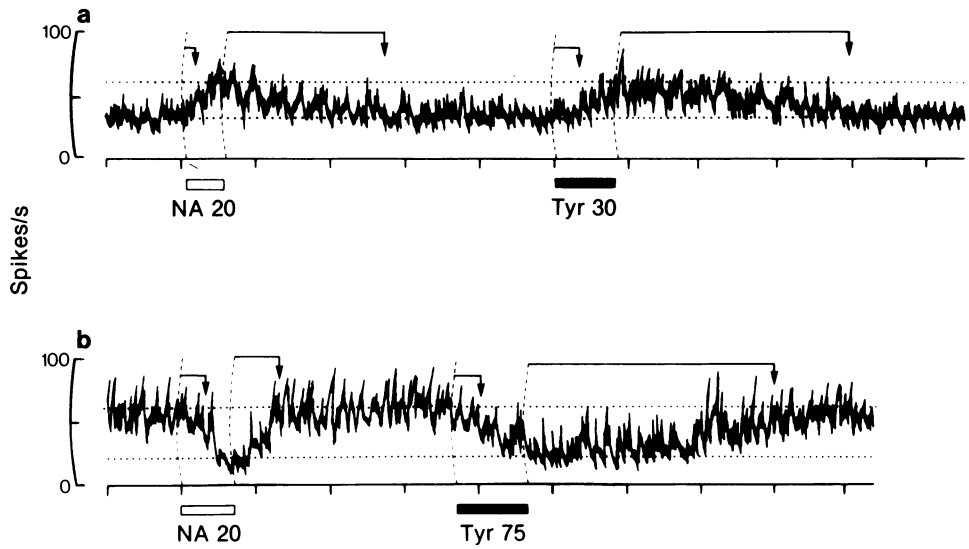

Figure 2 Comparison of time courses of responses to tyramine and noradrenaline. Ratemeter recordings of the firing rates of two cortical neurones (a and b) (as in Figure 1). Horizontal dotted lines correspond to the levels of the baseline firing rates and to the maximum increases or decreases in firing rate attained by the cells in response to the agonists. Vertical broken lines correspond to the onsets and offsets of ejecting pulses. Horizontal bars with arrows above the traces indicate time-course parameters; from the bottom: latency to onset; recovery time. (a) A cell which was excited by both tyramine (Tyr) and noradrenaline (NA). Both drugs evoked approximately equivalent maximum changes in firing rate; the time course parameters (latency to onset, recovery time), however, were greater for the response to tyramine than for the response to noradrenaline. (b) A cell which was depressed by both tyramine and noradrenaline. Both drugs evoked approximately equivalent maximum changes in firing rate; the time-course parameters, however, were greater for the response to tyramine than for the response to noradrenaline.

obtained, desipramine was applied continuously either by removal of the retaining current (i.e. 0 nA) thus allowing the drug to diffuse out from the micropipette, or by the passage of a weak ejecting current (5 to 10 nA) and the time course of the developing antagonism or potentiation was followed. The response to an agonist was regarded as antagonized if there was a reduction of more than 50% in the overall size ('total spike number') of the response. Similarly the response to an agonist was regarded as potentiated if there was an increase of more than 50% in the overall size of the response. ('Total spike number' is defined as the total number of action potentials generated in response to an agonist application; Bradshaw *et al.*, 1973b).

Excitatory responses Drug-interaction studies were successfully completed on 47 cortical neurones excited by both tyramine and NA. On 41 cells, desipramine could discriminate between responses to tyramine and NA: on all these cells the response to tyramine was abolished, while the response to NA was either potentiated (11 cells) or unaffected (30 cells). On the remaining 6 neurones, the excitatory responses to tyramine and NA were equally antagonized. DL-Homocysteic acid was used as a control agonist; responses to this drug were not affected by desipramine. Recovery of responses to tyramine from the effects of desipramine usually was very slow, taking on occasions as long as 2 h to develop. On a few cells full recovery of the responses could not be observed even after several hours. An example of the effects of desipramine on excitatory responses to tyramine and NA is shown in Figure 3.

Depressant responses The effects of desipramine were examined on 10 cells depressed by both tyramine and NA. On 8 of these cells, the response to tyramine was antagonized, while the response to NA was either potentiated (5 cells) or unaffected (3 cells) by desipramine; see Figure 4. On the remaining 2 cells, responses to both amines were equally antagonized.

Comparison of the transport numbers of tyramine and noradrenaline

The transport numbers of tyramine and NA were compared using 8 micropipettes; the results are shown in Table 2. The mean transport number of tyramine obtained from the 8 micropipettes was

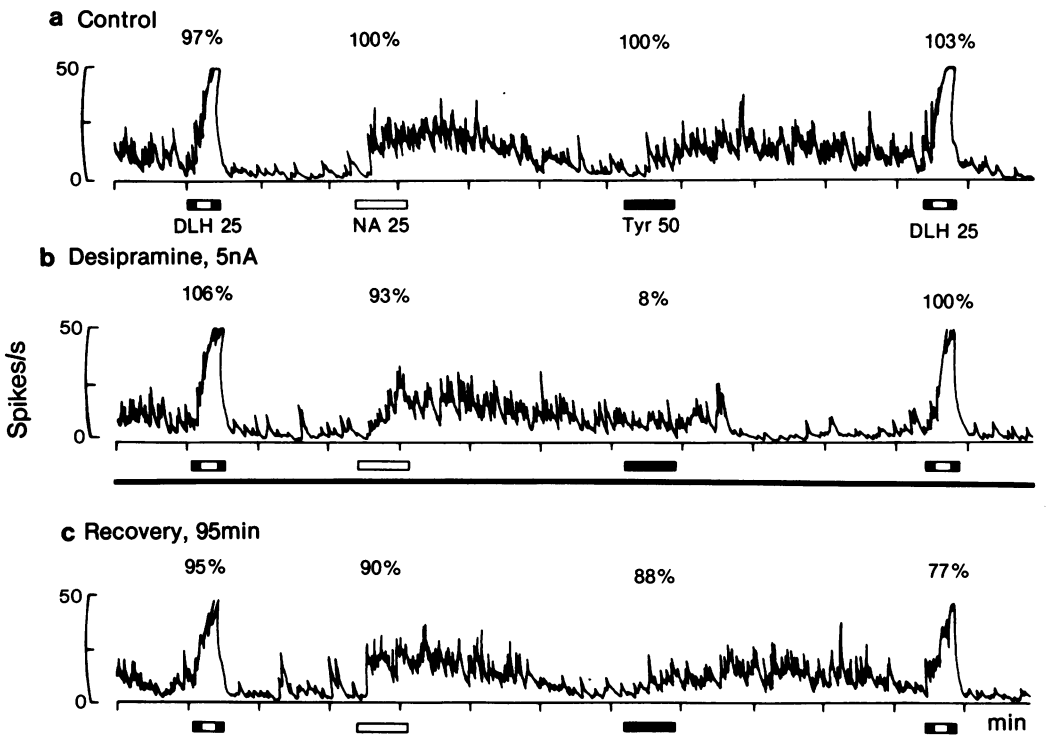

Figure 3 Effects of desipramine on excitatory responses to tyramine noradrenaline, and DL-homocysteic acid. Ratemeter recording of the firing rate of a single cortical neurone (as in Figure 1). Figures above the traces indicate total spike numbers (%), taking the sizes of the mean of the control responses to each agonist as 100%. (a) Control responses to tyramine (Tyr), noradrenaline (NA), and DL-homocysteic acid (DLH). (b) Responses to the agonists during the continuous application of desipramine. The application of desipramine is indicated by the thick continuous line below the trace. At the start of trace (b) desipramine (5 nA) had been applied continuously for 20 min. The response to tyramine was antagonized, whereas the responses to noradrenaline and DL-homocysteic acid were unaffected. (c) Recovery of the response to tyramine 95 min after the application of desipramine had been terminated.

0.336 ± 0.030, whereas the mean transport number of NA was 0.283 ± 0.025. There was no significant difference between the transport numbers of tyramine and NA (paired t test; $P = 0.115$).

Discussion

Tyramine and NA had similar agonistic effects on cortical neurones: the vast majority of the neurones responded in the same direction to the two amines. When compared on the same neurones, tyramine appeared to have a lower potency than NA. Since there was no significant difference between the transport numbers of tyramine and NA, it is probable that the observed difference in potency reflects a genuine biological difference between the two drugs (see Curtis, 1964; Bradshaw & Szabadi, 1974). The lower potency of tyramine may reflect an indirect action of the amine, since it has been reported that tyramine does not displace NA stoichiometrically from adrenergic terminals (Axelrod, Gordon, Hertting, Kopin & Potter, 1962). It is noteworthy that the transport number of NA obtained in these experiments is somewhat higher than has generally been reported previously (see Kelly, 1975); however, similar values have been obtained in another recent study conducted in our laboratory (Bevan, Bradshaw, Pun, Slater & Szabadi, 1978b).

The responses to tyramine had a slower time course than responses to NA. This is consistent with an indirect action of tyramine, since time would be required for the tyramine molecules released from the micropipette to reach the NA-containing nerve terminals, to be taken up by the terminals, to displace and release NA, and for the released NA molecules to reach the postsynaptic receptors.

Desipramine could discriminate between neuronal responses to tyramine and NA: while responses to tyramine were antagonized, responses to NA were

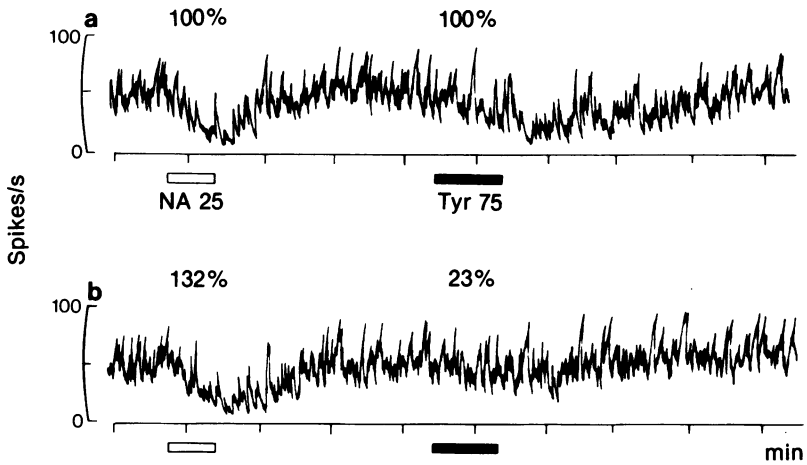

Figure 4 Effects of desipramine on depressant responses to tyramine and noradrenaline. Ratemeter recording of the firing rate of a single cortical neurone (as in Figures 1 and 3). (a) Control responses to tyramine (Tyr) and noradrenaline (NA). (b) Responses to the agonists 63 min after a prolonged application of desipramine (10 nA for 25 min). The response to tyramine was antagonized, whereas the response to NA was not significantly affected.

potentiated or unaffected. The most plausible explanation for this observation is the blockade of the NA-uptake mechanism by desipramine: uptake blockade could prevent tyramine from reaching the presynaptic stores of NA, and thus could prevent the pharmacological response; on the other hand, uptake blockade could result in potentiation of the response to NA by interfering with the major process of removal of NA from postsynaptic receptor sites (see Iversen, 1974).

Although potentiation of the response to NA could sometimes be observed when the response to tyramine was antagonized, on many occasions no potentiation of the response to NA could be seen (see Figures 3 and 4). This observation may reflect the postsynaptic adrenoceptor blocking properties of desipramine (see Sturman, 1970; McCulloch & Story, 1972; Bradshaw *et al.*, 1971; 1974). It is possible that, in some experiments, the blockade of postsynaptic receptors by desipramine prevented the development of the potentiation of responses to NA which would have resulted from the presynaptic uptake blockade.

Table 2 Transport numbers of noradrenaline and tyramine obtained from eight six-barrelled micropipettes

Pipette	Tip diameter (μm)	Transport number* noradrenaline	tyramine
1	4.0	0.292 ± 0.013	0.391 ± 0.025
2	3.5	0.229 ± 0.046	0.460 ± 0.049
3	5.0	0.257 ± 0.015	0.248 ± 0.013
4	4.0	0.409 ± 0.012	0.432 ± 0.014
5	4.0	0.250 ± 0.016	0.275 ± 0.009
6	4.0	0.197 ± 0.009	0.253 ± 0.011
7	4.0	0.255 ± 0.003	0.265 ± 0.006
8	5.0	0.377 ± 0.008	0.367 ± 0.014
		Paired t test: $P = 0.115$	

* Values are mean ± s.e. mean of 16 observations.

The present results are compatible with the hypothesis that there is a close relationship between uptake blockade and potentiation of neuronal responses to NA. However, it has been reported that desipramine can potentiate neuronal responses to NA, and to other monoamines, in situations where uptake blockade is unlikely to operate (Bevan, Bradshaw & Szabadi, 1975a, b; 1976). Thus, although uptake blockade may result in potentiation, its operation does not seem to be a pre-requisite for potentiation (see Bradshaw *et al.*, 1974).

The present results suggest that tyramine may act by releasing NA from NA-containing nerve terminals in the brain. Indeed, there is evidence that tyramine is accumulated in brain tissue by an active process which is inhibited by desipramine (Ross & Renyi, 1966), and that it can displace NA from brain synaptosomes (Colburn & Kopin, 1972). However, the possibility cannot be excluded that tyramine may release not only NA, but also other monoamine transmitters such as dopamine or 5-hydroxytryptamine. Furthermore, our results do not eliminate the possibility that tyramine may directly activate postsynaptic receptors, as has been suggested by Hoffer *et al.* (1971).

This work was supported by the North Western Regional Health Authority and the Mental Health Foundation. We are grateful to Mrs E. Markham for technical assistance.

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(*Received January 28, 1978. Revised February 27, 1978.*)