all. Since it may be assumed that Ca^{2+} influx into the terminal nerve fibres increases with increasing $CaCl_2$ concentrations used for stimulation[7], modulation of Ca^{2+} availability for stimulus-release coupling appears to be the more effective, the smaller the transmembrane inward current of Ca^{2+} ions. When the Ca^{2+} influx exceeds a certain amount, no modulation can take place. This may be due to the fact that variations of the intraneuronal Ca^{2+} concentration near the saturation level of the Ca^{2+} receptors which trigger ^{3}H-noradrenaline release will cause no alteration of release[6]. Under the present conditions (α-adrenoceptor-mediated modulation blocked) the Ca^{2+} influx induced by $CaCl_2$ 1.3 mmoles/l may be assumed to be of such a magnitude that the intraneuronal Ca^{2+} receptors appear to be fully occupied.

In conclusion, the present results provide evidence that modulation of noradrenaline release from cortical noradrenergic nerve fibres, caused by interaction of morphine with presynaptic opiate receptors, is mediated by decreasing the availability of Ca^{2+} ions for stimulus-release coupling. This suggestion is in agreement with the finding that the injection of morphine decreases the Ca^{2+} concentration in various regions of the brain[9,10] and in brain synaptosomes[11,12]. Interestingly, brain calcium has been shown to play an important role in the analgesic action of morphine[13].

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Effects of tranylcypromine on the concentrations of some trace amines in the diencephalon and hippocampus of the rat[1]

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Summary. Concentrations of 4 trace amines in diencephalon and hippocampus of the rat were measured by integrated-ion-current mass spectrometry after administration of the antidepressant drug, tranylcypromine. Much larger increases were observed for 2-phenylethylamine and tryptamine than for m- and p-tyramine.

The term 'trace amines'[4] has been applied to a number of substances (e.g. 2-phenylethylamine (PE), tryptamine (T), m- and p-tyramine (TA), octopamine and phenylethanolamine) which are present in brain tissue at concentrations much lower than those of the catecholamines and 5-hydroxytryptamine (5-HT). The rapid turnover rates of the trace amines[5-7] compared to those of the classical putative neurotransmitter amines, as well as the fact that two of them, PE and T, pass the blood-brain barrier with ease[8], suggest that the trace amines may be functionally important. It has been proposed that a functional deficiency of PE[9,10] or T[9] in the CNS may be involved in the etiology of depression. Urinary excretion of these amines has been reported to be lower in depressed patients than in controls[11,12]. It has been shown that treatment of depressed patients with the monoamine oxidase (MAO) inhibitor phenelzine results in a marked increase in the urinary excretion of T[9]. Dramatic increases of PE and/or T have been reported in whole brain[13-15], as well as in striatum, hypothalamus, cerebellum and brain stem[16,17] after administration of MAO inhibitors.

In view of the action of antidepressant drugs and the suggested involvement of PE and T in depression, we felt it would be useful to study the effects of tranylcypromine (TCP), a clinically efficacious antidepressant drug with strong MAO inhibiting properties, on the concentrations of these 2 amines in the diencephalon and hippocampus of the rat. For comparative purposes, m- and p-TA were assayed concomitantly in these brain areas since Tabakoff et al.[14] have reported that administration of TCP results in much larger increases of T than of p-TA in mouse whole brain.

The diencephalon is the cephalic end of the reticular-activating system and the hippocampus is a component of the limbic system. These areas, which are interrelated through the Papez circuit[18], are thought to play an important role in the regulation of mood. They are known to influence components of depressive illness such as disturbances of sleep, arousal, appetite, sexual behavior, motivation and memory, as well as the output of the cerebral cortex itself[19].

Methods. Male Wistar rats weighing 200–240 g were injected i.p. with TCP hydrochloride (20 mg/kg). After periods of time ranging from 0.25 to 6 h, the rats were killed, the brains removed and the hippocampus and diencephalon dissected out and immediately frozen in isopentane on solid carbon dioxide. Brain regions from individual TCP-treated rats or from 3 control rats were then homogenized in ice-cold sodium carbonate solution (15% w/v) containing 25 ng of each of the deuterated amines (m-TA, p-TA, PE and T) as internal standards. The samples were then extracted and dansylated, and the dansylamines separated

Effect of tranylcypromine on trace amine concentrations in rat hippocampus and diencephalon

Brain region	Treatment	m-Tyramine	p-Tyramine	Tryptamine	Phenylethylamine
Hippocampus	Control	0.44±0.15 (7)	1.29±0.19 (9)	1.45±0.08 (5)	1.55± 0.38 (8)
	TCP, 0.25 h	0.41±0.08 (5)	2.26±0.22 (5)**	5.81±0.36 (6)***	76.80± 7.3 (5)***
	TCP, 0.75 h	1.72±0.12 (4)***	2.67±0.22 (5)***	10.90±0.6 (6)***	146.0 ±11.0 (5)***
	TCP, 1.5 h	1.50±0.13 (6)***	4.07±0.55 (9)***	38.1 ±2.9 (9)***	214.0 ±42.0 (9)***
	TCP, 6 h	1.33±0.14 (5)**	1.49±0.29 (6)	43.6 ±8.1 (7)***	104.0 ± 7.0 (7)***
Diencephalon	Control	0.44±0.11 (5)	1.26±0.12 (7)	0.7± 0.18 (4)	1.97± 0.39 (7)
	TCP, 0.25 h	0.81±0.02 (5)*	3.08±0.17 (5)***	12.4± 0.7 (6)***	72.5 ± 3.7 (5)***
	TCP, 0.75 h	1.92±0.04 (5)***	4.31±0.11 (5)***	25.2± 1.2 (6)***	111.0 ± 7.0 (5)***
	TCP, 1.5 h	1.85±0.16 (6)***	7.34±0.70 (9)***	58.5± 4.0 (9)***	191.0 ±39.0 (9)***
	TCP, 6 h	1.98±0.12 (3)***	3.35±0.47 (6)**	52.4±12.0 (6)**	104.0 ±18.0 (6)***

Values are expressed as ng/g of wet tissue and represent mean±SEM, with the number of experiments shown in parentheses. Student's t-tests were performed to determine the significance of the difference between the drug treated values and control values: *$p<0.05$; **$p<0.01$; ***$p<0.001$.

by TLC and quantitated by integrated ion current mass spectrometry as described by Philips and Boulton[17].

Results and discussion. The results, shown in the table, demonstrate that in the diencephalon and hippocampus the increases in concentrations of PE and T are much greater than those of the tyramines after administration of TCP. The greatest increases found for each of the amines were as follows: m-TA, 4.5-fold; p-TA, 5.8-fold; PE, 138-fold; and T, 84-fold. The concentrations of m-TA and T, which increased for 0.75 h and 1.5 h, respectively, after TCP injection, changed very little thereafter until at least 6 h after the drug. However, p-TA and PE, which also attained their maximum concentrations at 1.5 h, declined markedly between 1.5 h and 6 h. By 6 h, p-TA in the hippocampus had returned to control values. The parallel increase and decline in the levels of PE and p-TA is interesting since it has been reported that PE can be hydroxylated in the phenyl ring to form p-TA in the rat[20,21]. This has been proposed as the major route of p-TA formation, while dehydroxylation of catechols has been suggested as the primary synthetic route for m-TA[22].

The results obtained here would seem to support the suggestion that PE and T may play a role in the action of MAO-inhibiting antidepressant drugs. The increases seen are much larger and more rapid than those observed in the concentrations of the putative neurotransmitter amines, dopamine (DA), noradrenaline (NA), and 5-HT under the same circumstances. In a preliminary study[23], we have shown that 1.5 h after a 20 mg/kg dose of TCP, significant increases in the concentrations of DA and 5-HT occurred in the diencephalon, but that these increases were less than 100% above control values. NA concentrations showed a small but significant decrease from control values, but there was a marked increase in normetanephrine levels, perhaps reflecting the reported ability of TCP to inhibit NA re-uptake and to release NA in brain tissue[24,25]. What effects these increases in PE and T may have on CNS function is not clear at this time. However, at the concentrations attained in vivo in the present study, PE and T are able to alter uptake and release of the catecholamines and/or 5-HT in brain tissue in vitro[26,27]. These actions, along with the apparent direct effects of PE and T on central receptors[28], suggest that these amines may well alter neuronal functioning in the CNS and may be an important component in the pharmacological activity of TCP.

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