No.	R^1	R^2	R^3	m.p. (°C)	Nidation inhibition ED_{50} (mg/kg) s.c.	p.o.
1	H	H	H	204–206	>4	>4
2	OH	H	H	202–203	0.5	0.5
3	Acetyloxy	H	H	158–160	>4	–
4	5-Bromonicotinoyloxy	H	H	185–187	1–2	1
5	Isonicotinoyloxy	H	H	158–161	2–4	2–4
6	Hexanoyloxy	H	H	135–137	1–2	0.5–1
7	Benzoyloxy	H	H	164–166	1	0.5
8	Pivaloyloxy	H	H	155–156	1–2	0.5–1
9	2,6-Dimethoxybenzoyloxy	H	H	142–146	2–4	2–4
10	2,6-Dimethylbenzoyloxy	H	H	184–186	4	2
11	Benzoyloxy	H	Br	200–202	2	4
12	Benzoyloxy	H	Cl	198–200	0.7	1.5
13	Benzoyloxy	CH_3	H	168–170	>16	9
14	Ethoxy	H	H	121–123	>4	–
15	Fenoxy	H	H	128–130	>4	>4
16	Benzoylthio	H	H	165–167	>4	>4
17	2-Bromo-α-ergocryptine				1.3	7.7

methylation[10] of compound 7 with CH_3J and $NaNH_2$ in liq. NH_3.

Pharmacology. The antiprolactin activity of the derivatives has been indirectly evaluated utilizing the nidation inhibition test in rats[11], prolactin being the single pituitary hormone responsible for the function of the corpora lutea in early pregnancy in this species[12]. Pregnant Sprague-Dawley rats weighing 200–250 g were used. The compounds to be tested, suspended in 5% acacia gum, were administered p.o. or s.c. on day 3 of pregnancy (day 1 = day of sperms or plug detection).

On day 14 the animals were anesthetized and the uteruses were examined for the presence of implantation sites.

Each substance was administered at the screening dose of 4 mg/kg to groups of 5–7 rats: the active compounds were tested at lower doses for the approximate ED_{50} evaluation.

2-Bromo-α-ergocryptine well known for both its anti-nidation and anti-prolactin activity[13] has been used as reference standard.

The results are summarized in the table. These data suggest that, with the notable exception of compound 3, all the esters are hydrolyzed in vivo to the active ketol (No. 2), although an activity per se of the esters cannot be excluded. Compounds No. 1, 14, 15, 16 that cannot yield the ketol No. 2 are inactive at the screening dose level. Halogenation in position 2 does not modify the antiprolactin activity whereas methylation in position 1 practically abolishes it.

On the basis of these data and of the stability tests, the benzoate (No. 7) has been selected for further investigation.

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Inhibition of Ca^{2+}-induced noradrenaline release from central noradrenergic neurons by morphine

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Summary. Morphine inhibited the noradrenaline release from slices of rat brain cortex induced by introduction of Ca^{2+} ions after superfusion with Ca^{2+}-free, K^+-rich solution. The degree of inhibition was inversely related to the Ca^{2+} concentration used for stimulation.

Morphine[2,3] and the endogenous compounds enkephalin[3,4] and β-endorphin[5] inhibit the noradrenaline release from rat brain slices evoked by electrical pulses or high extracellular K^+ concentration; the inhibition is probably mediated by opiate receptors, since it was antagonized by naloxone. These receptors appear to be located on the varicosities of the terminal noradrenergic fibres[3], and it has been suggested that their activation may decrease the availability of Ca^{2+} ions for stimulus-release coupling[6]. On the basis of these suggestions we studied whether the noradrenaline release induced by introduction of Ca^{2+} ions after superfusion of brain cortex slices with Ca^{2+}-free, K^+-rich solution is decreased by morphine and whether the degree of inhibition is dependent on the Ca^{2+} concentration used for

stimulation. Noradrenaline release evoked by this method of stimulation is probably due to Ca^{2+} influx via the potential-sensitive Ca^{2+} channel of the cell membrane[7]. Our data provide further evidence for the hypothesis that morphine decreases the availability of Ca^{+} for stimulus-release coupling.

Methods. Occipital cortex slices (diameter 3.0 mm, 0.3 mm thickness) of male Wistar rats weighing 200–300 g were incubated in physiological salt solution containing 10^{-7}M (−)-^{3}H noradrenaline (sp. act. 555 GBq/mmole; Amersham-Buchler, Braunschweig) for 60 min. This solution was composed as follows (in mmole/l): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, ascorbic acid 0.06, disodium EDTA 0.03, glucose 10 (aeration with 95% O_2 and 5% CO_2; 37 °C). Subsequently, the slices were superfused with Ca^{2+}-free solution containing K^+ 30 mmoles/l (isomolar replacement of NaCl by KCl) at a rate of 0.5 ml/min. For stimulation with Ca^{2+} ions, $CaCl_2$ was introduced into this solution for 2 min, 40 and 80 min after onset of superfusion (S_1 and S_2). The radioactivity in the superfusates and slices was determined by liquid scintillation counting. All details of the methods used and of calculation of Ca^{2+}-evoked tritium overflow above basal efflux have been described elsewhere[7].

Results and discussion. In all experiments phentolamine 1 µmole/l and cocaine 10 µmoles/l were present throughout superfusion in order to abolish the α-adrenoceptor-mediated modulation[6,8] and to inhibit neuronal uptake and intraneuronal metabolism of ^{3}H-noradrenaline, respectively. In the presence of cocaine, the Ca^{2+}-evoked tritium overflow has been shown to consist almost exclusively of ^{3}H-noradrenaline[7]. Therefore, under these conditions the Ca^{2+}-induced ^{3}H overflow may be denoted as ^{3}H-noradrenaline release. Introduction of $CaCl_2$ 0.16 to 1.3 mmoles/l for 2 min after superfusion of cortex slices with Ca^{2+}-free, K^+-rich solution evoked a concentration-dependent ^{3}H-noradrenaline release (figure 1).

When ^{3}H-noradrenaline release was stimulated twice with $CaCl_2$ 0.33 mmoles/l after 40 (S_1) and 80 min (S_2) of superfusion, the ratio of the release evoked by S_2 and that evoked by S_1 amounted to 0.88 ± 0.11 (N = 6). In the presence of morphine (20 min before and during S_2) the Ca^{2+}-induced ^{3}H-noradrenaline release was inhibited in a concentration-dependent manner: At morphine concentrations of 0.1, 1 and 10 µmoles/l, the ratio S_2/S_1 amounted to 0.62 ± 0.10 (N = 7), 0.48 ± 0.08 (N = 8; $p < 0.02$, compared to the controls) and 0.38 ± 0.07 (N = 6; $p < 0.005$), respectively. Naloxone which, given alone, did not alter the Ca^{2+}-induced ^{3}H-noradrenaline release antagonized the inhibitory effect of morphine (figure 2), indicating that the latter is mediated by opiate receptors.

Interestingly, the degree of inhibition caused by morphine was inversely related to the Ca^{2+} concentration used for stimulation (figure 3). Whereas the ^{3}H-noradrenaline release evoked by the lowest Ca^{2+} concentration of 0.16 mmoles/l was diminished by more than 70%, the release evoked by Ca^{2+} 1.3 mmoles/l was not modified at

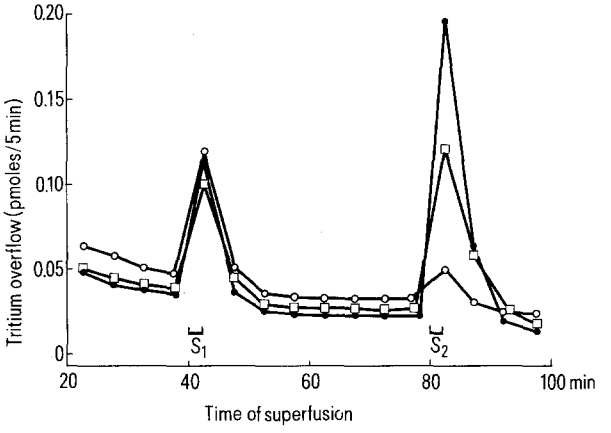

Fig. 1. Efflux of tritium from cortex slices in the presence of phentolamine 1 µmole/l and cocaine 10 µmoles/l throughout superfusion. Means (for clarity, SEM has been omitted). From 0 to 100 min the slices were superfused with Ca^{2+}-free buffer containing K^+ 30 mmoles/l and Na^+ 119 mmoles/l; tritium overflow was stimulated twice (S_1 and S_2) by introduction of $CaCl_2$ for 2 min. The $CaCl_2$ concentration used for S_1 was 0.33 mmoles/l in all experiments, whereas that used for S_2 was 0.16 (○, N = 6), 0.65 (□, N = 5) or 1.3 mmoles/l (●, N = 6); at these Ca^{2+} concentrations the ratio of the tritium overflow (above basal efflux) evoked by S_2 and that evoked by S_1 amounted to (means ± SEM) 0.31 ± 0.06, 2.55 ± 0.12 and 3.84 ± 1.08, respectively.

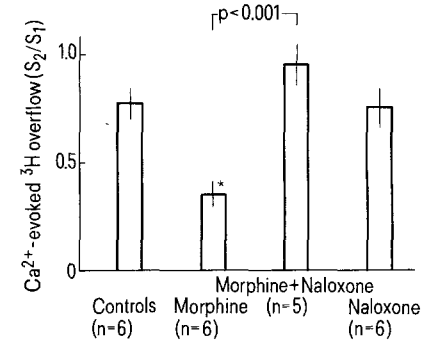

Fig. 2. Effects of morphine 1 µmole/l, naloxone 10 µmoles/l and simultaneous administration of the drugs on tritium overflow from cortex slices evoked by introduction of $CaCl_2$ 0.33 mmoles/l for 2 min after superfusion with Ca^{2+}-free buffer containing K^+ 30 mmoles/l and Na^+ 119 mmoles/l. Phentolamine 1 µmole/l and cocaine 10 µmoles/l were present throughout superfusion. Tritium overflow which was stimulated twice, 40 (S_1) and 80 min (S_2) after onset of superfusion, was expressed as the ratio of the overflow evoked by S_2 and that evoked by S_1 (S_2/S_1; means ± SEM). The drugs were present in the superfusion medium from 20 min before S_2 until the end of the experiments. *$p < 0.01$ (compared to the controls).

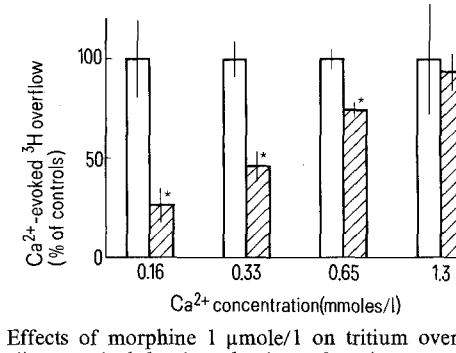

Fig. 3. Effects of morphine 1 µmole/l on tritium overflow from cortex slices evoked by introduction of various concentrations of $CaCl_2$ after superfusion with Ca^{2+}-free buffer containing K^+ 30 mmoles/l and Na^+ 119 mmoles/l. Phentolamine 1 µmole/l and cocaine 10 µmoles/l were present throughout superfusion. Tritium overflow was stimulated twice, 40 (S_1) and 80 min (S_2) after onset of superfusion. The $CaCl_2$ concentration used for S_1 was 0.33 mmoles/l in all experiments, whereas that used for S_2 varied from 0.16 to 1.3 mmoles/l (abscissa). Ordinate: Evoked tritium overflow (ratio of the overflow evoked by S_2 and that evoked by S_1) expressed as percent of the corresponding controls. Open columns: control experiments (ratios of S_2/S_1 corresponding to 100% are given in figure 2 and legend of figure 1). Hatched columns: experiments with morphine (present from 20 min before S_2 until the end of the experiments). Means ± SEM (N = 5 or 6). *$p < 0.01$ (compared to the corresponding controls).

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