collected and prepared for analysis. Cerebral blocks of brain were cut out, rinsed with buffered solution and perfused structures were dissected out and frozen in liquid nitrogen. Perfusates and brain extracts were desalted and deproteinized using methods of ion exchange displacement[2,3] and a modification of the picric acid method[4] and were then subjected to automated amino-acid analysis. Eluate fractions from the analyser were collected for 1 min intervals and appropriate aliquots were assayed for radioactivity. Endogenous and labelled dopamine and norepinephrine were determined by previously described methods[5-7].

Table 1. CONTENTS AND SPECIFIC RADIOACTIVITIES OF AMINO-ACIDS IN AMYGDALA AND PUTAMEN OF RHESUS MONKEYS

Amino-acid	Brain extract: Mole/g ($\times 10^6$)	Brain extract: d.p.m. in 10^{-6} moles	Perfusate: d.p.m./ml. collection fluid
Monkey No. 2 (perfusion of left amygdala with labelled glucose)			
α-Alanine	1·49	2,364	195
Aspartic acid	0·84	640	—
GABA	2·06	1,165	170
Glutamic acid	8·42	1,523	420
Glutamine, serine + asparagine	3·50	693	0
Glycine	1·14	—	0
Monkey No. 6 (perfusion of left amygdala with labelled γ-HBA)			
α-Alanine	0·43	274	—
Aspartic acid	1·25	—	—
GABA	1·45	664	—
Glutamic acid	8·45	84	0
Glutamine, serine + asparagine	5·18	941	0
Glycine	0·80	0	—
Monkey No. 6 (perfusion of right putamen with labelled γ-HBA)			
α-Alanine	0·70	449	—
Aspartic acid	0·88	—	—
GABA	1·10	—	—
Glutamic acid	9·48	153	0
Glutamine, serine + asparagine	5·30	985	0
Glycine	0·96	—	—

The specific radioactivities were calculated assuming that the total area perfused was excised and was constant in each case, and that each sample was equivalent in weight. Abbreviations: 0, less than $0{\cdot}2 \times 10^{-9}$ mole of amino-acid or less than 20 d.p.m.; —, not determined usually because of overlap with other peaks of radioactivity. The very high value for α-alanine content in monkey No. 2 is presumed to be caused by relative hypoxia in the area which was perfused. The values for the combined peak of glutamine, serine-plus-asparagine were calculated using the constants which were determined for serine only.

Table 1 shows that with labelled glucose as precursor, glutamic acid, GABA and α-alanine acquired considerable radioactivity and that these compounds were released in labelled form into the perfusion fluid. Though the studies with labelled γ-hydroxybutyric acid also gave evidence of labelling of GABA and glutamic acid, the large combined peak of glutamine, serine-plus-asparagine contained most of the radioactivity of extracts; the perfusates of these areas contained radioactive amino-acids in amounts comparable with those found with glucose, but the emergence of several large peaks of radioactivity (ninhydrin negative metabolites of γ-hydroxybutyric acid) during the collection of the first 30–40 ml. of effluent volume precluded determination of radioactivity under most peaks.

Table 2. NEWLY SYNTHESIZED CATECHOLS AND CATECHOLAMINES IN AMYGDALA AND PUTAMEN OF RHESUS MONKEYS; INDIVIDUAL VALUES

Substance formed	Total d.p.m. in the region excised: Left amygdala, Monkey No. 7	Left amygdala, Monkey No. 8	Right putamen, Monkey No. 7	Right putamen, Monkey No. 8
Total radioactivity	309,722	456,304	1,631,713	304,694
Catechols	3,268	9,073	388,585	41,951
Dopamine	1,700	1,248	267,639	27,755
Norepinephrine	348	402	747	444

Table 2 shows the results obtained in structures of monkey brain perfused with labelled tyrosine. The putamen has a considerably greater capacity for the synthesis of total catechols and dopamine than the amygdala of monkeys, while there seems to be no difference between the capacity of these areas to synthesize norepinephrine. The total dopamine and norepinephrine contents of these areas in three other monkeys were found to be $64{\cdot}5 \pm 7{\cdot}8$ and $1{\cdot}6 \pm 0{\cdot}2 \times 10^{-9}$ moles, respectively, for the putamen and $3{\cdot}7 \pm 1{\cdot}1$ and $1{\cdot}4 \pm 0{\cdot}1 \times 10^{-9}$ moles, respectively, for the amygdala (*S.E.*, five determinations). Apparently very efficient uptake mechanisms exist for these catecholamines because none of the newly formed amines could be collected in the perfusates. The possibility exists, however, that the labelled amines may have been metabolized before their exit into the perfusion fluid.

In light of these results, it now seems possible to monitor the release of newly formed pools of pharmacologically active amino-acids from various structures of the brains of chronically implanted monkeys while these animals are awake and being exposed to different situations which might modify their behaviour.

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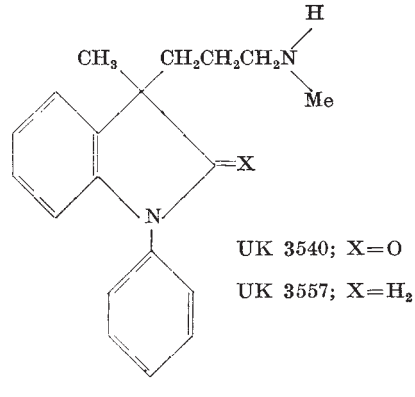
N-Phenyl Indoline Derivatives—a New Class of Antidepressant Agents

THE discovery in 1957 of the therapeutic effect of imipramine in endogenous depression[1] stimulated a search for other substances with similar properties, and several drugs which are structurally closely related to imipramine, known collectively as the tricyclic antidepressants, have become available[2]. The spectrum of antidepressant effects of these drugs is fairly uniform, although some possess tranquillizing activity, which is useful for treating patients whose depression is complicated by agitation[3,4]. But these substances have side effects which stem partly from anticholinergic and antihistaminic properties[5]. In the past 5 yr attempts have been made to depart from the tricyclic structures, and antidepressant activity has been claimed for iprindole[6], IN 1060 (ref. 7), thiazesim[8] and other compounds, but it is too early to assess their place in the therapy of depression.

Here we report attempts to develop an antidepressant drug of novel structure which might display new pharma-



UK 3540; X=O

UK 3557; X=H_2

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cological and clinical features. Medicinal chemical considerations led to the development of a series of aminoalkyl-substituted N-phenyl indolines and 2-indolinones. The pharmacological activity of these substances suggested possible antidepressant properties. Two compounds of outstanding interest, Pfizer UK 3540 and Pfizer UK 3557, were selected for extensive investigation. Full details of structure–activity relationships will be described elsewhere.

They were synthesized by treatment of 3-methyl-1-phenyl-2-indolinone[9] with 3-(N-benzyl-N-methylamino)-propyl chloride in the presence of sodamide. Hydrogenolysis of the product gave UK 3540, and this was converted to UK 3557 by reduction with diborane.

Both compounds prolonged and potentiated the pressor effect of adrenaline and noradrenaline in cats anaesthetized with chloralose and caused a marked increase in the contractions of the nictitating membrane evoked by electrical stimulation of the preganglionic cervical sympathetic nerve. They were potent antagonists of the hypothermia induced in mice either by intracerebral injection of noradrenaline or by subcutaneous injection of reserpine. They also antagonized in rats the sedation induced by intraperitoneal injection of tetrabenazine. Oral administration (1 mg/kg) to conscious dogs had no effect on blood pressure or heart rate *per se*, but the magnitude and duration of pressor responses to noradrenaline were potentiated, while the pressor responses to tyramine were abolished. They inhibited *in vitro* and *in vivo* the uptake of tritiated noradrenaline in rat brain and heart. Neither compound showed any evidence of monoamine oxidase inhibition either *in vitro*, using homogenized livers from animals pretreated with UK 3540 or UK 3557, or *in vivo*, as judged by failure to induce forelimb convulsions after intravenous administration of tryptamine in predosed rats.

These properties of UK 3540 and UK 3557 are shown to varying extents by the tricyclic antidepressants. The two new compounds do not, however, possess the antihistaminic and anticholinergic activities of imipramine and its congeners, as judged by pA_2 values measured in isolated guinea-pig ileum preparations; it is these two properties of substances of the tricyclic group which are thought to contribute to their clinical side effects[5]. Preliminary results of clinical evaluations of UK 3540 and UK 3557 are encouraging.

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Enlargement of Spinal Cord Synapses after Repetitive Stimulation of a Single Posterior Root

Although it has frequently been suggested that repetitive stimulation may result in a structural change which would increase the "efficiency" of the synapse[1,2], there is little histological evidence for this, even though Cragg[3] has demonstrated synaptic changes in the visual cortex of the rat after exposure to light. Whether or not sensory stimuli can bring about a structural change in the central nervous system (CNS) raises a particular problem of synaptic plasticity which is interesting with regard to post-tetanic potentiation, CNS connectivity, learned behaviour and regeneration in the CNS.

I report here the appearance of structures in the spinal cord after repetitive afferent stimulation compared with the normal condition and with the giant synapses usually found in Clarke's column.

Adult cats were anaesthetized with intraperitoneal 'Nembutal'. A catheter in the left ventricle was connected by a two way tap to normal saline and fixation material[4]. The left fifth lumbar root was exposed by enlarging the root foramen without disturbing the spinal cord or the dura mater, cardiac pacemaker electrodes were placed around the posterior root, and the root was stimulated with a 1 ms pulse, 300/s, 2·5 V. Stimulation continued for 65 min and then perfusion fluid was run in during stimulation (two cats), 5 min after stimulation had stopped (two cats), and 90 min after stimulation (two cats). In a control experiment I repeated this procedure without stimulation and left the operation site exposed for more than an hour before perfusion. After perfusion, spinal segments of the stimulated root and C7 segment (as a control) were prepared and stained by the method of Armstrong and Stephens[4]. Sections were cut 8 microns thick. Boutons were measured from photographs (× 1,000) taken at the same time as a picture of a substage micrometer. Boutons in contact with both cell body and dendrite were measured along their longest axis. For comparison with Clarke's column boutons, two adult cats were killed and L4 segments were prepared as described.

After stimulation of a single posterior root (L5) for 65 min, changes were seen in the areas where monosynaptic fibres are known to terminate[5–9]. The distribution of these changes was the same in all cats and was much more marked on the ipsilateral side. No abnormal changes were seen in sections taken from the cervical cord in the stimulated animals and no changes were seen in the control animal with the site of the operation exposed for 1 h. The density of changes after stimulation was very much less than the density of monosynaptic endings in these areas. In the sections studied there was no disruption of the bouton mosaic characteristic of the normal situation, and in particular none of the early changes of degeneration[10] were seen. The abnormal boutons were larger than normal (Table 1) and were mostly oval or spherical with a smooth outline and, like the normal, an internal structure could be discerned even by light microscopy. These larger boutons were usually darker staining than normal, and the internal structure, as far as could be made out using the light microscope, was made up of several dark-staining particles the size of the (presumed) mitochondria seen in the normal boutons and several much smaller particles at the limit of resolution.

Table 1

Size-groups (microns)	Normal animal		Post-stimulation (measurements from mid-zone and anterior horn)		t	Clarke's column	
	No.	Per cent (n = 6)	No.	Per cent (n = 6)		No.	Per cent (n = 5)
< 2	1,098	91·5 ± 6·3	1,223	69·9 ± 5·2	$P > 0·001$	325	65 ± 5·4
2–4·8	95	8·0 ± 5·7	388	22·5 ± 4·1	$P > 0·001$	114	23 ± 6·7
More than 4·8	7	0·6 ± 0·8	136	7·5 ± 1·6	$P > 0·001$	61	12 ± 3·3

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