ANTIDEPRESSANTS AND RECEPTOR FUNCTION

A Wiley – Interscience Publication

1986

JOHN WILEY & SONS

Chichester New York Brisbane Toronto Singapore
ANTIDEPRESSANTS AND RECEPTOR FUNCTION
The Ciba Foundation is an international scientific and educational charity. It was established in 1947 by the Swiss chemical and pharmaceutical company of CIBA Limited—now CIBA-GEIGY Limited. The Foundation operates independently in London under English trust law.

The Ciba Foundation exists to promote international cooperation in biological, medical and chemical research. It organizes about eight international multidisciplinary symposia each year on topics that seem ready for discussion by a small group of research workers. The papers and discussions are published in the Ciba Foundation symposium series. The Foundation also holds many shorter meetings (not published), organized by the Foundation itself or by outside scientific organizations. The staff always welcome suggestions for future meetings.

The Foundation's house at 41 Portland Place, London, W1N 4BN, provides facilities for meetings of all kinds. Its Media Resource Service supplies information to journalists on all scientific and technological topics. The library, open seven days a week to any graduate in science or medicine, also provides information on scientific meetings throughout the world and answers general enquiries on biomedical and chemical subjects. Scientists from any part of the world may stay in the house during working visits to London.
Contents

Symposium on Depression, antidepressants and receptor sensitivity, held at the Ciba Foundation, London, 19–21 November 1985

Editors: Ruth Porter, Gregory Bock (Organizers) and Sarah Clark

D. Murphy  Introduction  1

S.Z. Langer, A.M. Galzin, C.R. Lee and H. Schoemaker  Antidepressant-binding sites in brain and platelets  3
Discussion  17

Discussion  36

P. Propping, W. Friedl, J. Hebebrand and K-U. Lentes  Genetic studies at the receptor level: investigations in human twins and experimental animals  42
Discussion  50

M. Åsberg and A. Wägner  Biochemical effects of antidepressant treatment—studies of monoamine metabolites in cerebrospinal fluid and platelet [³H]imipramine binding  57
Discussion  77

R.W. Horton, C.L.E. Katona, A.E. Theodorou, A.S. Hale, S.L. Davies, C. Tunnicliffe, Y. Yamaguchi, E.S. Paykel and J.S. Kelly  Platelet radioligand binding and neuroendocrine challenge tests in depression  84
Discussion  96

D.L. Murphy, C.S. Aulakh and N. A. Garrick  How antidepressants work: cautionary conclusions based on clinical and laboratory studies of the longer-term consequences of monoamine oxidase-inhibiting antidepressants  106
Discussion  120
CONTENTS

S.A. Checkley, T.H. Corn, I.B. Glass, C. Thompson, C. Franey and J. Arendt
Neuroendocrine and other studies of the mechanism of antidepressant action of desipramine  126
Discussion  142

L.J. Siever, E.F. Coccaro, E. Benjamin, K. Rubinstein and K.L. Davis
Adrenergic and serotonergic receptor responsiveness in depression  148
Discussion  159

General discussion I  164

A. Frazer, G. Ordway, J. O'Donnell, P. Vos and B. Wolfe  Effect of repeated administration of clenbuterol on the regulation of β-adrenoceptors in the central nervous system of the rat  170
Discussion  183

J.M. Weiss and P.G. Simson  Depression in an animal model: focus on the locus ceruleus  191
Discussion  209

G.W. Kraemer  Causes of changes in brain noradrenaline systems and later effects on responses to social stressors in rhesus monkeys: the cascade hypothesis  216
Discussion  227

J. Vetulani, L. Antkiewicz-Michaluk, A. Rokosz-Pelc and J. Michaluk  Effects of chronically administered antidepressants and electroconvulsive treatment on cerebral neurotransmitter receptors in rodents with 'model depression'  234
Discussion  241

A.R. Green, D.J. Heal and G.M. Goodwin  The effects of electroconvulsive therapy and antidepressant drugs on monoamine receptors in rodent brain —similarities and differences  246
Discussion  260

General discussion II  268

Closing remarks  278

Index of contributors  281

Subject index  283
Participants

M. Åsberg  Department of Psychiatry, Karolinska Hospital, Box 60500, S-104 01 Stockholm, Sweden

W.H. Berrettini  Section on Clinical Genetics, Clinical Neurogenetics Branch, National Institute of Mental Health, Bldg 10, Room 3N220, National Institutes of Health, Bethesda, MD 20892, USA

A. Biegon  (Ciba Foundation Bursar) Isotope Department, The Weizmann Institute of Science, Rehovot 76100, Israel

D.S. Charney  Clinical Research Unit, Abraham Ribicoff Research Facilities, Dept of Psychiatry & Connecticut Mental Health Center, Yale University School of Medicine, 34 Park Street, New Haven, CT 06508, USA

S.A. Checkley  Maudsley Hospital, Denmark Hill, London SE5 8AZ, UK

A.J. Coppen  MRC Neuropsychiatry Research Laboratory, West Park Hospital, Epsom, Surrey KT19 8PB, UK

A. Delini-Stula  Biology Research, Clinical Psychopharmacology Unit, CIBA-GEIGY AG, CH-4002 Basel, Switzerland

A. Frazer  Neuropsychopharmacology Unit, Department of Psychiatry, University of Pennsylvania, c/o VA Hospital (151E), University and Woodland Aves, Philadelphia, PA 19104, USA

R.W. Fuller  Eli Lilly & Co, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA

A.R. Green  Astra Neuroscience Research Unit, c/o Institute of Neurology, National Hospital, Queen Square, London WC1N 3BG, UK

R.W. Horton  Department of Pharmacology and Clinical Pharmacology, St George’s Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, UK
J.S. Kelly  Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, UK

G.W. Kraemer  Behavioral Psychopharmacology Unit, Primate Laboratory, University of Wisconsin, 22 North Charter Street, Madison, WI 53715, USA

S.Z. Langer  Département de Recherche Biologique, Laboratoires d'Etudes et de Recherches Synthélabo (LERS), 58 rue de la Glacière, 75013 Paris, France

D.L. Murphy  (Chairman) Laboratory of Clinical Science, National Institute of Mental Health, NIH Clinical Center, 10-3D41, 9000 Rockville Pike, Bethesda, MD 20892, USA

E.S. Paykel  Department of Psychiatry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QO, UK

R.D. Porsolt  34 rue de la Porte Jaune, 92100 Saint Cloud, France

R. Porter  6 Raglan Street, Kentish Town, London NW5 3DA, UK

P. Propping  Institut für Humangenetik der Universität Bonn, Wilhelmstrasse 31, 5300 Bonn 1, Federal Republic of Germany

L.J. Siever  Out-patient Psychiatry Clinic, Department of Psychiatry, 116a, Bronx Veterans Administration Medical Center, 130 West Knightsbridge Road, Bronx, NY 10468, USA

F. Sulser  Tennessee Neuropsychiatric Institute, Department of Pharmacology, Vanderbilt University School of Medicine, 1501 Murfreesboro Road, Nashville, TN 37217, USA

T.H. Svensson  Department of Pharmacology, Karolinska Institute, PO Box 60 400, S-104 01 Stockholm, Sweden

J. Vetulani  Department of Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31-343 Cracow, Poland

J.M. Weiss  Neurobehavioral Research Laboratory, Department of Psychiatry, Duke University Medical Center, Box 3829, Durham, NC 27710, USA
Introduction

D.L. MURPHY

Laboratory of Clinical Science, National Institute of Mental Health, NIH Clinical Center, 10-3D41, 9000 Rockville Pike, Bethesda, MD 20892, USA

1986 Antidepressants and receptor function. Wiley, Chichester (Ciba Foundation Symposium 123) p 1–2

First, a word of appreciation to Gene Paykel and Ruth Porter, who persuaded the Ciba Foundation of the potential value of this symposium, and then inspired the participants to come to London for this meeting. Antidepressant drugs alter brain neurotransmitter receptors, and we shall hear a series of formal papers describing these receptor changes. Perhaps more importantly though, the format of this symposium will provide more time for informal discussion, critique and, I hope, reformulation of this burgeoning area in psychopharmacology, with a view towards future studies.

β-Adrenoceptor down-regulation by antidepressant treatments, originally reported by Vetulani & Sulser (1975), was a germinal finding that led to the discovery of many more time-dependent alterations in the activity of neurotransmitter systems during longer-term administration of antidepressant drugs. The basis for the receptor changes, their dependence on changes in other neurotransmitter pathways and their ultimate functional significance remain open areas for investigation.

The symposium will begin with several papers evaluating a notion still in its infancy: the possibility that changes in central neurotransmitter receptors, and perhaps related adaptational events, occur as part of the process of becoming depressed, or are present as vulnerability factors, genetic or otherwise, for affective illness. An implied corollary of this notion is that the consequences of antidepressant treatments in individuals with depression may differ from those in normal humans—or normal rats. This brings us to the necessity of developing, where possible, suitable animal models for studying depression and antidepressant drug treatments and, of course, the ultimate necessity of evaluating hypotheses regarding receptor alterations in humans.

Monoamine, hormone and peptide receptors have become a focal point for much current research in pharmacology, and it is striking how even a single drug exposure can alter a receptor site for the lifetime of an organism. Not all
time-dependent adaptational events are necessarily related to receptor changes, and we will need to keep distinct what can be learned from changes in the numbers of ligand-binding sites and the ultimate changes in physiological functions or behaviour. The question of how antidepressant treatments work has been much aided and stimulated by the discovery of brain receptor mechanisms, but we are certainly a long and challenging way from understanding the process by which different chemical molecules act to ameliorate human anxiety, depression and despair.

REFERENCE

Vetulani J, Sulser F 1975 Actions of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain. Nature (Lond) 257:495-496
Antidepressant-binding sites in brain and platelets

S.Z. LANGER, A.M. GALZIN, C.R. LEE and H. SCHOEMAKER

Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo (L. E. R. S.), 58 rue de la Glacière, 75013 Paris, France

Abstract. [3H]Imipramine and [3H]paroxetine label with high affinity a site associated with the serotonin transporter in brain and platelets. The maximum binding capacity ($B_{max}$) of [3H]imipramine in platelets is reduced in untreated depressed patients, and it may represent a useful biological marker in depression. The existence of an endogenous ligand acting on the [3H]imipramine-recognition site to modulate the serotonin transporter has been proposed by several laboratories. 5-Methoxytryptoline inhibits [3H]imipramine binding and [3H]serotonin uptake in the nanomolar range. This compound has been reported to occur in the pineal gland, but probably only in trace amounts. While the physiological relevance of 5-methoxytryptoline or a close analogue remains an open question, the possibility exists that the 'endocoid' for the [3H]imipramine-recognition site plays a role in the pathogenesis of depression.


Most antidepressant drugs of well established clinical efficacy either inhibit the neuronal uptake of noradrenaline or serotonin or act on both monoamine systems. Yet the antidepressant efficacy of these drugs requires a latency period of two to three weeks, in spite of the fact that neuronal uptake of noradrenaline or serotonin (5-hydroxytryptamine, 5-HT) may be fully inhibited within the first day or two of drug administration. This phenomenon has aroused special interest in the modulation of neurochemical and behavioural variables during chronic administration of antidepressants and in the molecular mechanisms underlying such effects.

In attempts to identify specific high affinity binding sites that could be related to the mechanism of inhibition of serotonin or noradrenaline uptake, [3H]imipramine and [3H]desipramine have been used as radioligand probes. There is now substantial evidence that, while [3H]imipramine labels with high affinity a site associated with the serotonin transporter in brain and platelets (Langer et al 1980a,b, 1981a,c), [3H]desipramine labels a site associated with

In serotonergic neurons and in platelets, several ligands of different chemical structures (the tricyclic drug [3H]imipramine, and the non-tricyclic drugs [3H]-paroxetine and [3H]indalpine) bind with high affinity to the recognition site associated with the 5-HT transporter complex. This recognition site mediates inhibition of the Na⁺-dependent uptake of 5-HT, which indicates that it is much more than a non-specific drug acceptor site. Dissociation kinetic experiments support the view that the substrate-recognition site for 5-HT within the 5-HT transporter complex is different from the receptor labelled by [3H]imipramine (Segonzac et al 1985). Consequently, the [3H]imipramine-recognition site may represent a novel type of presynaptic receptor whose function is to modulate 5-HT uptake. These and other results lead to the suggestion that an ‘endocoid’ may exist which acts on [3H]imipramine-recognition sites to modulate the uptake of 5-HT (Langer & Raisman 1983, Langer et al 1984b, Barbaccia et al 1983, Barbaccia & Costa 1984).

The possible existence of an endogenous ‘imipramine-like’ substance may be relevant to the aetiology of affective disorders and to the clinical finding by several laboratories of a decrease in the density of platelet [3H]imipramine-binding sites in untreated severely depressed patients (Langer et al 1981c, 1984b, Lewis & McChesney 1985). Similarly, a decrease in the density of [3H]imipramine-binding sites has been reported in post-mortem brains from patients with depressive illness (Perry et al 1983).

**Methods**

Membranes from human and rabbit platelets or from the rat brain were prepared according to the methods described by Langer et al (1980a,b) and Raisman et al (1980). [3H]Imipramine binding was measured according to the methodology described by Raisman et al (1980).

**Results and discussion**

The high affinity binding site for [3H]imipramine has been found in membranes prepared from various regions of the brain of all species examined to date, including humans (Langer et al 1981c, 1982, Raisman et al 1979a,b, 1980). [3H]Imipramine also binds saturably and with high affinity to membranes prepared from blood platelets (Briley et al 1979, Langer et al 1980a). The high affinity [3H]imipramine-binding site possesses most of the characteristics of a pharmacological receptor, as has been reviewed extensively (Langer et al 1981c).

The high affinity binding of [3H]imipramine is potently inhibited by tricyclic antidepressants and by non-tricyclic inhibitors of the neuronal uptake of 5-HT (Table 1). 5-Hydroxytryptamine is the only neurotransmitter that inhibits
Drug inhibition of the 5-HT transporter in human platelets was studied with 0.6 nM-[3H]imipramine at an incubation temperature of 0 °C. Each Ki value represents the mean of at least three different experiments in which nine concentrations of the compound were tested in duplicate.

[3H]imipramine binding, but it is not as potent as the drugs that inhibit 5-HT uptake: the concentration of 5-HT required for half-maximal inhibition of [3H]imipramine binding (IC50) is in the low micromolar range (Raisman et al 1980), whereas the IC50 values for some tricyclic antidepressants and other non-tricyclic inhibitors of 5-HT uptake are in the nanomolar range (Table 1, cf. Sette et al 1983).

The association of [3H]imipramine binding with the transporter for the 5-HT uptake mechanism in serotonergic nerve endings has been clearly established (Brunello et al 1982, Gross et al 1981, Langer et al 1980a,b, Sette et al 1981). It is likely that [3H]imipramine labels a physiologically relevant site that modulates 5-HT uptake (Langer et al 1983), rather than a simple recognition site for tricyclic compounds. One piece of evidence for this is that tritiated non-tricyclic inhibitors of 5-HT uptake like [3H]norzimeldine (Hall et al 1982) and [3H]paroxetine (Habert et al 1985) label with high affinity the same site as that labelled by [3H]imipramine.

Moreover, our results suggest that, although the site labelled by [3H]imipramine is associated with the 5-HT transporter system, it may be different from the substrate-recognition site for 5-HT (Langer et al 1983) (Fig. 1). Thus, [3H]imipramine binds with high affinity to a presynaptic site that modulates the neuronal uptake of 5-HT, in analogy with the presynaptic autoreceptors that modulate the release of their neurotransmitter (Langer 1981). It should be pointed out, however, that the release-modulating presynaptic autoreceptors are acted upon by the neurotransmitter itself (Langer 1981), whereas the presynaptic receptor involved in the modulation of 5-HT uptake may be acted upon by a novel endogenous substance. This endogenous factor may be of humoral origin or be released locally, either as a cotransmitter with 5-HT or from adjacent nerve terminals. The existence of such an endogenous ligand, different from 5-HT and acting on the [3H]imipramine-binding site, was suggested recently (Langer et al 1983, Langer et al 1982, Sette et al 1983). Additional extensive work is necessary to establish the presence and clarify the possible physiological role of this postulated endocoid.

### Table 1 Inhibition of [3H]imipramine-binding in human platelets

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ki (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipramine</td>
<td>2.6</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>3.1</td>
</tr>
<tr>
<td>Desipramine</td>
<td>25.5</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>1.7</td>
</tr>
<tr>
<td>Femoxetine</td>
<td>23.3</td>
</tr>
<tr>
<td>Citalopram</td>
<td>6.0</td>
</tr>
<tr>
<td>5-Methoxytryptoline</td>
<td>22.0</td>
</tr>
</tbody>
</table>

Drug inhibition of the 5-HT transporter in human platelets was studied with 0.6 nM-[3H]imipramine at an incubation temperature of 0 °C. Each Ki value represents the mean of at least three different experiments in which nine concentrations of the compound were tested in duplicate.
FIG. 1. Schematic representation of the possible relationship between the binding site for \(^{3}\text{H}\)imipramine (\(^{3}\text{H}\)-IMI) and the serotonin uptake mechanism. The possible sites of interaction of 5-HT indicated are the postsynaptic 5-HT\(_1\) receptor, the postsynaptic 5-HT\(_2\) receptor, the presynaptic 5-HT autoreceptor, which regulates the release of 5-HT by a negative feedback mechanism, and the 5-HT uptake complex with which the high affinity \(^{3}\text{H}\)imipramine-binding site is associated. Two different recognition sites appear to be present for the transporter of 5-HT on the nerve terminals: the substrate-recognition site for 5-HT and the \(^{3}\text{H}\)-IMI receptor, which may be a modulatory unit for the uptake of 5-HT. The \(^{3}\text{H}\)-IMI-binding site may be a target for an endogenous ligand different from 5-HT.

A recent report on the occurrence in rat brain of a specific endogenous inhibitor of \(^{3}\text{H}\)imipramine binding and \(^{3}\text{H}\)-5-HT uptake is of most interest in this context (Barbaccia et al. 1983). Although the hypothesis is very speculative, we cannot exclude the possibility that this endocoid can act as a neuromodulator on \(^{3}\text{H}\)imipramine-binding sites, regardless of whether or not they are coupled to the transporter of 5-HT.

We have recently investigated structure–activity relationships among known indole amines and their derivatives, some of which occur endogenously. Among these substances, we were particularly interested in the tryptolines, which preferentially inhibit the uptake of 5-HT (Buckholtz & Boggan 1976, 1977, Kellar et al. 1976, Airaksinen et al. 1980).

As shown in Fig. 2, both tryptamine and 5-HT inhibit in the low micromolar range the binding of \(^{3}\text{H}\)imipramine at an incubation temperature of 0 °C to membranes from human platelets, while 5-methoxytryptamine is considerably less potent. Melatonin is completely inactive (data not shown). It is interesting that in membranes from the rat brain cortex 5-HT and tryptamine are equipotent at inhibiting the binding of \(^{3}\text{H}\)imipramine and \(^{3}\text{H}\)paroxetine (Langer et al. 1984a, Habert et al. 1985). Yet in the solubilized transporter complex labelled with \(^{3}\text{H}\)paroxetine, there is a 10-fold decrease in the potency of tryptamine at
ANTIDEPRESSANT-BINDING SITES

FIG. 2. Structure–activity relationship for the inhibition of [3H]imipramine binding by tryptamine and tetrahydro-β-carbolines (tryptolines). The structures of the tryptamines and tryptolines are shown, with the 5-hydroxy and 5-methoxy substitutions. IC_{50} values are the concentrations of compounds required to inhibit 50% of the specific binding of [3H]imipramine to human platelet membranes, determined at 0.6 nM-[3H]imipramine. Each value shown is the mean of at least three experiments in which the compound was tested at nine concentrations in duplicate.

inhibiting [3H]paroxetine binding (Habert et al 1986), although the potency of 5-HT remains unchanged.

In the tryptoline series, the structure–activity relationship is inverted with respect to the corresponding indole amines (Fig. 2). Here, 5-methoxytryptoline is the most potent at inhibiting [3H]imipramine binding to human platelet membranes, and the unsubstituted compound is the least potent. Similar structure–activity relationships apply to the affinities of indole amines and tryptolines for [3H]imipramine-recognition sites in the rat brain (Langer et al 1984b, Barbaccia & Costa 1984).

When the tryptolines are tested on either [3H]imipramine or [3H]paroxetine binding at temperatures higher than 0°C, a clear reduction in potency is observed (Schoemaker et al 1986). At 22°C or 37°C, the IC_{50} values for the tryptolines at inhibiting [3H]paroxetine binding correspond to their potencies at inhibiting 3H-5-HT uptake, determined also at 37°C (Schoemaker et al 1986, Segonzac et al 1986). In human platelets, 5-methoxytryptoline is the most potent of the tryptolines tested at inhibiting 3H-5-HT uptake, so the order of
potency is the same as for the inhibition of \([^3\text{H}]\text{imipramine binding (data not shown).}

In the rat hypothalamus and suprachiasmatic nucleus regions, 5-HT uptake undergoes important circadian changes (Meyer & Quay 1976). Uptake of \(^3\text{H}\)-5-HT peaks near the onset of darkness, and is at its minimum near the onset of light (Meyer & Quay 1976). The suprachiasmatic nucleus has a very high serotonergic innervation and plays a major role in the generation and maintenance of normal circadian rhythms (Rusak & Zucker 1979). It is tempting to speculate that changes in the local concentration of an imipramine-like endocoid may be related to the reported circadian rhythm of \(^3\text{H}\)-5-HT uptake in these regions. Consistent with this view is the fact that there is also a circadian rhythm in the density of \([^3\text{H}]\text{imipramine-binding sites in the suprachiasmatic nucleus of the rat (Wirz-Justice et al 1983). This phenomenon may be significant in view of the hypothesis that internal desynchronization of circadian rhythms might be causally related to depression (for review, see Wehr & Goodwin 1981).}

Several important points have to be clarified before the suggestion can be advanced that 5-methoxytryptoline or a closely related analogue modulates the neuronal uptake of 5-HT. The first point concerns the occurrence of 5-methoxytryptoline in certain tissues like the adrenal gland, the brain, the retina and the pineal gland. Secondly, it is important to demonstrate a mechanism for the inactivation of 5-methoxytryptoline, either catabolism to inactive compounds, or reuptake through an active transport mechanism. Thirdly, the complete profile of physiological and pharmacological actions of 5-methoxytryptoline, in addition to the inhibition of 5-HT uptake and \([^3\text{H}]\text{imipramine binding, will have to be described. For this, the development of selective antagonist drugs that could specifically block the physiological and pharmacological effects of 5-methoxytryptoline would be of great value.}

The determination of tissue levels of tryptolines is controversial because of shortcomings in the methods available to the analyst. Significant levels of 5-methoxytryptoline have been reported in the retinae and pineal glands of several species (Kari 1981, Kari et al 1983, Leino et al 1983). These are the organs which contain hydroxyindole \(\text{O}\)-methyltransferase, an enzyme likely to be essential for the synthesis of 5-methoxytryptoline (see below).

We have recently examined the presence of 5-methoxytryptoline in the pineal glands of several species, using a method that can control for its artifactual formation (Langer et al 1985). Our results indicate considerably lower levels of 5-methoxytryptoline than those reported earlier by Kari (1981), Kari et al (1983), Leino et al (1983) and Langer et al (1984c). It appears that, at best, 5-methoxytryptoline is present in trace amounts in the pineal gland of the species examined.

Tryptophan is the most likely precursor for the tryptolines. Aromatic-L-amino-acid decarboxylase and tryptophan 5-hydroxylase are both extensively
distributed, and therefore the 5-hydroxylated and decarboxylated derivatives of tryptophan are readily available to many tissues. However, the biosynthesis of 5-methoxytryptoline is likely to be confined to tissues containing hydroxyindole O-methyltransferase, like the pineal gland and the retina.

The main problem in the synthetic pathway for 5-methoxytryptoline involves the additional carbon atom required for the closing of the third cycle. This could be derived in vivo from formaldehyde (Leysen & Laduron 1974) or from a one-carbon-substituted tetrahydrofolate.

In relation to the inactivation of 5-methoxytryptoline, Airaksinen et al (1978) reported that the tritiated compound was taken up by rabbit platelets. However, recent experiments in our laboratories clearly indicate that, although 3H-5-methoxytryptoline is accumulated by rabbit platelets, it is not actively transported through the platelet membrane (A. Segonzac, H. Schoemaker & S.Z. Langer, unpublished observations) or into rat hypothalamic slices (A.M. Galzin & S.Z. Langer, unpublished observations). We have found that the temperature-dependent accumulation of 3H-5-methoxytryptoline is not Na+ dependent or inhibited by ouabain, but is most likely related to differences in the rate of 3H-5-methoxytryptoline diffusion into platelets at 0 °C and 37 °C. Consequently, an active transport of 5-methoxytryptoline is unlikely to be a route of inactivation for this compound. An enzymic pathway is more probable (Ho et al 1972): the major catabolic route is by hydroxylation in the 6-position followed by conjugation.

When infused into the third ventricle of the rat at doses as low as 4 nmol and 20 nmol, 5-methoxytryptoline produces behavioural stimulation and desynchronization in electrocortical activity with a decrease in total voltage power (Nistico et al 1986). In the same model, 5-hydroxytryptoline is eight times less potent than 5-methoxytryptoline, while the unsubstituted tryptoline is practically inactive (Nistico et al 1986).

In relation to the suggested neuroendocrine role for 5-methoxytryptoline (Kari et al 1983, Langer et al 1984), the effects of this compound and some of its analogues on plasma levels of different hormones are of particular interest. In rats, 5-methoxytryptoline increases plasma prolactin levels (Smythe et al 1983, Rovescalli et al 1986). Increased plasma adrenocorticotropic hormone and corticosterone levels have also been reported, as has a decrease in growth hormone release (Smythe et al 1983).

The neurochemical effects of 5-methoxytryptoline include an increase in brain concentrations of 5-HT and a reduction in those of the deaminated metabolite 5-hydroxyindoleacetic acid (Airaksinen & Kari 1981). These effects of 5-methoxytryptoline are most likely related to the inhibition of neuronal uptake of 5-HT, but they may in addition reflect the ability of this compound to inhibit monoamine oxidase activity at high doses.

As already mentioned, 5-methoxytryptoline inhibits serotonin uptake and [3H]imipramine and [3H]paroxetine binding in brain and platelets. However,
the compound is practically inactive (in concentrations up to 10 µM) as an inhibitor of radioligand binding to other receptors and recognition sites: α- and β-adrenoceptor subtypes, dopamine receptors, 5-HT₁ and 5-HT₂ receptors, opioid receptors and binding sites for [³H]diazepam, [³H]cocaine and [³H]tryptamine.

The possibility that the pharmacological effects of 5-methoxytryptoline involve a different specific '5-methoxytryptoline receptor' cannot be discounted, but it is highly speculative. With the use of ³H-5-methoxytryptoline, a high affinity binding site with an equilibrium dissociation constant (K_D) of 50 nM has been identified in the rat cerebral cortex (D. Graham, C. Lee & S.Z. Langer, unpublished observations). Nevertheless, its pharmacological properties remain to be characterized; in particular, there is no information concerning possible antagonists. The increase in plasma prolactin levels induced in rats by high doses of 5-methoxytryptoline is mediated by indirect activation of postsynaptic 5-HT receptors, because it is blocked by the antagonists metergoline and cyproheptadine (Rovescalli et al 1986).

Platelets have an active transport mechanism for 5-HT which resembles the neuronal uptake mechanism for 5-HT in the brain, including the modulatory receptor labelled with high affinity by [³H]imipramine (Briley et al 1982, Langer et al 1980a). The high affinity [³H]imipramine-binding site in blood platelets appears to be identical to that found in the brain of various species, including humans, and may be considered a potential model for the binding site in the brain. As shown in Table 2, our laboratory has demonstrated a significantly lower maximum binding capacity (B_max) for [³H]imipramine in platelets from untreated, severely depressed patients than in platelets from control volunteers; the K_D values are normal in the depressed group (Briley et al 1980). Other studies have also demonstrated a lower density of [³H]imipramine-binding sites in the platelets of untreated, severely depressed patients than in normal control volunteers matched for age and sex (Table 2). However, one study (Mellerup et al 1982) shows that the B_max of [³H]-imipramine in platelets is slightly (but statistically significantly) higher in depressed manic melancholic patients than in control groups (Table 2). This discrepancy may be due to differences in patient populations or diagnostic criteria or to methodological differences in the determination of [³H]imipramine binding. Furthermore, five recent clinical studies failed to detect differences in the B_max of [³H]imipramine in platelets between depressed untreated patients and control volunteers (Table 2). Nevertheless, the fact remains that in 10 out of the 16 studies (Table 2) there is a significantly lower B_max of [³H]imipramine in the platelets of depressed, untreated patients than in the platelets of controls, without a concomitant difference in the K_D values.

As previously suggested, changes in the binding of [³H]imipramine in human platelets may reflect those occurring in the brain (Briley et al 1982, Langer et al 1982). In support of this view, it was recently demonstrated that the B_max values
for \(^{3}H\)imipramine in the post-mortem brains of people who had committed suicide and depressed patients were significantly lower than those of appropriate controls (Perry et al 1983, Stanley et al 1982).

Our longitudinal studies indicate that the density of \(^{3}H\)imipramine-binding sites in human platelets does not change during treatment with tricyclic antidepressant drugs, in spite of a major improvement in psychiatric parameters (Raisman et al 1981). In addition, severely depressed patients treated with maprotiline, the selective inhibitor of noradrenaline uptake, did not show any change in the low \(B_{\text{max}}\) value for \(^{3}H\)imipramine in platelets, in spite of the clinical recovery from the depression obtained during the treatment (Raisman et al 1981).

One possible explanation for these results was that the continued presence of tricyclic antidepressant drugs prevented a return of the \(B_{\text{max}}\) values to the higher control levels, because in both brain and platelets, chronic antidepressant treatment has been shown to result in a significant reduction of the \(B_{\text{max}}\) for \(^{3}H\)imipramine (Briley et al 1982). However, we have recently reported that after at least six sessions of electroconvulsive therapy and without other antidepressant medication, the \(B_{\text{max}}\) value for \(^{3}H\)imipramine in platelets from severely endogenously depressed patients remained low, and did not differ

---

**TABLE 2** Different studies of \(^{3}H\)imipramine binding in platelets from untreated depressed patients and control volunteers matched for age and sex

<table>
<thead>
<tr>
<th>Reference</th>
<th>(B_{\text{max}}) for (^{3}H)imipramine (% difference between depressed patients and controls)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Briley et al (1980)</td>
<td>-54%</td>
</tr>
<tr>
<td>Asarch et al (1981)</td>
<td>-22%</td>
</tr>
<tr>
<td>Paul et al (1981)</td>
<td>-29%</td>
</tr>
<tr>
<td>Mellerup et al (1982)</td>
<td>+ 9%</td>
</tr>
<tr>
<td>Suranyi-Cadotte et al (1982)</td>
<td>-54%</td>
</tr>
<tr>
<td>Baron et al (1983)</td>
<td>NS</td>
</tr>
<tr>
<td>Egrise et al (1983)</td>
<td>NS</td>
</tr>
<tr>
<td>Langer &amp; Raisman (1983)</td>
<td>-43%</td>
</tr>
<tr>
<td>Whitaker et al (1984)</td>
<td>NS</td>
</tr>
<tr>
<td>Suranyi-Cadotte et al (1985)</td>
<td>-26%</td>
</tr>
<tr>
<td>Lewis &amp; McChesney (1985)</td>
<td>-40%</td>
</tr>
<tr>
<td>Benneftat et al (1985)</td>
<td>-47%</td>
</tr>
<tr>
<td>Schneider et al (1985)</td>
<td>-20%</td>
</tr>
<tr>
<td>Wagner et al (1985)</td>
<td>-10%</td>
</tr>
<tr>
<td>Gentsch et al (1985)</td>
<td>NS</td>
</tr>
<tr>
<td>Tang &amp; Morris (1985)</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a\)The \(B_{\text{max}}\) of \(^{3}H\)imipramine in platelets was compared in depressed untreated patients and control volunteers matched for age and sex. Significant differences between the two groups are given as percentages; NS, non-significant differences. Note that 10 out of 16 studies reported significantly lower \(B_{\text{max}}\) values for \(^{3}H\)imipramine in platelets from depressed untreated patients.
significantly from the corresponding values obtained before treatment, in spite of the clinical improvement (Langer et al. 1986).

These results suggest that the density of [3H]imipramine-binding sites in platelets may be a 'state-independent' rather than 'state-dependent' biological marker in depression. In other words, the low density of [3H]imipramine-binding sites in platelets may reflect a vulnerability or a genetic susceptibility to depression. The latter may be of considerable interest, because it is generally agreed that a genetic predisposition exists in a substantial proportion of patients with manic-depressive illness. This index of chronic constitutional vulnerability should, therefore, be expected to be present in euthymic monopolar or bipolar patients, when compared with normal control individuals. In this regard, Berrettini et al. (1982) have reported no difference between 12 medication-free euthymic bipolar patients and 12 normal volunteers in the $B_{\text{max}}$ or $K_D$ values of [3H]imipramine in platelets. However, the patients in this study had been treated with lithium before the two-week medication-free period, and long-lasting effects of lithium carbonate on the [3H]imipramine binding parameters in platelets cannot be excluded here. In addition, Berrettini et al. (1982) studied only bipolar patients, and there are no data from this group on euthymic monopolar patients.

In contrast with these results, we found in our most recent study that when depressed patients treated by electroconvulsive shock therapy were followed longitudinally for up to 18 months their $B_{\text{max}}$ values in platelets increased towards the range of normal values (Langer et al. 1986). These data are compatible with the view that [3H]imipramine binding in platelets is a state-dependent biological marker in depression, but that it recovers to normal values some time after the clinical improvement, probably when the remission is well consolidated. Additional studies in patients formerly depressed, but currently euthymic and unmedicated, are necessary to clarify whether [3H]imipramine binding is a state-independent or a state-dependent biological marker in depression.

The hypothesis that the density of [3H]imipramine-binding sites in platelets is genetically determined and is low in depression-prone individuals requires additional studies in large populations of depressed patients. Also, longitudinal studies in treated and in medication-free depressed patients are necessary. It is, however, of interest that a study by Paul et al. (1981) reported that the $B_{\text{max}}$ and the $K_D$ values of [3H]imipramine in platelets from pairs of monozygotic twins were practically identical, suggesting that [3H]imipramine binding in platelets may be genetically determined.

Conclusions

Both [3H]imipramine and [3H]paroxetine label with high affinity a recognition site associated with the macromolecular complex of the serotonin transporter
in brain and platelets. The site labelled by \([^3H]imipramine or[^3H]paroxetine possesses many of the properties of a pharmacological receptor, and may modulate serotonin uptake. The existence of an endogenous ligand acting on the \([^3H]imipramine-recognition site to modulate the uptake of 5-HT has been proposed by several laboratories. Among the possible candidates derived from tryptophan, 5-methoxytryptoline inhibits \([^3H]imipramine and \(^3H\)-5-HT uptake in nanomolar concentrations. There is a sharp structure–activity relationship for 5-methoxytryptoline, its 5-hydroxy analogue and the unsubstituted analogue in affinity for the \([^3H]\)imipramine-recognition site. Among nanomolar doses it may be present only in trace amounts. The physiological relevance of 5-methoxytryptoline or a close analogue as a possible endocoid for the \([^3H]\)imipramine-recognition site remains an open question.

The possible metabolic pathways for the synthesis and inactivation of 5-methoxytryptoline have been discussed. Although 5-methoxytryptoline has been reported to be present in such tissues as the pineal gland and the retina, it may be present only in trace amounts. The physiological relevance of 5-methoxytryptoline or a close analogue as a possible endocoid for the \([^3H]\)imipramine-recognition site remains an open question.

The possible metabolic pathways for the synthesis and inactivation of 5-methoxytryptoline have been discussed. Although 5-methoxytryptoline has been reported to be present in such tissues as the pineal gland and the retina, it may be present only in trace amounts. The physiological relevance of 5-methoxytryptoline or a close analogue as a possible endocoid for the \([^3H]\)imipramine-recognition site remains an open question.

The possible metabolic pathways for the synthesis and inactivation of 5-methoxytryptoline have been discussed. Although 5-methoxytryptoline has been reported to be present in such tissues as the pineal gland and the retina, it may be present only in trace amounts. The physiological relevance of 5-methoxytryptoline or a close analogue as a possible endocoid for the \([^3H]\)imipramine-recognition site remains an open question.

The possible metabolic pathways for the synthesis and inactivation of 5-methoxytryptoline have been discussed. Although 5-methoxytryptoline has been reported to be present in such tissues as the pineal gland and the retina, it may be present only in trace amounts. The physiological relevance of 5-methoxytryptoline or a close analogue as a possible endocoid for the \([^3H]\)imipramine-recognition site remains an open question.

The possible metabolic pathways for the synthesis and inactivation of 5-methoxytryptoline have been discussed. Although 5-methoxytryptoline has been reported to be present in such tissues as the pineal gland and the retina, it may be present only in trace amounts. The physiological relevance of 5-methoxytryptoline or a close analogue as a possible endocoid for the \([^3H]\)imipramine-recognition site remains an open question.
binding to platelets of depressed patients free of previous medication with 5-HT uptake inhibitors. Br J Pharmacol 85:215P
Briley MS, Langer SZ, Raisman R, Sechter D, Zarifian E 1980 Tiritated imipramine binding sites are decreased in platelets of untreated depressed patients. Science (Wash DC) 209:303–305
Buckholtz NS, Boggan WO 1977 Inhibition by 6-carbolines of monoamine uptake into a synaptosomal preparation: structure-activity relationships. Life Sci 20:2093–2100
ANTIDEPRESSANT-BINDING SITES

Langer SZ, Moret C, Raisman R, Dubocovich ML, Briley MS 1980b High-affinity $^3\text{H}$-imipramine binding in rat hypothalamus is associated with the uptake of serotonin but not norepinephrine. Science (Wash DC) 210:1133–1135


Langer SZ, Raisman R, Briley MS 1981b $^3\text{H}$-desipramine binding is associated with neuronal noradrenaline uptake in the periphery and the central nervous system. Eur J Pharmacol 72:423–424


Langer SZ, Sechter D, Loo H, Raisman R, Zarifian E 1986 Electroconvulsive shock therapy and Bmax of platelet $^3\text{H}$-imipramine binding in depression. Arch Gen Psychiatry, in press


Lewis DA, McChesney C 1985 Trinitrated imipramine binding distinguishes among subtypes of depression. Arch Gen Psychiatry 42:485–488


Mellerup C, Plenge P, Rosenberg R 1982 $^3\text{H}$-imipramine binding sites in platelets from psychiatric patients. Psychiatry Res 7:221–227


Paul SM, Rehavi M, Skolnick P, Ballenger JC, Goodwin FK 1981 Depressed patients
have decreased binding of $^3$H-imipramine to the platelets serotonin ‘transporter’.

Arch Gen Psychiatry 38:1315–1317


Raisman R, Sechter D, Briley MS, Zarifian E, Langer SZ 1981 High affinity $^3$H-imipramine binding in platelets from untreated and treated depressed patients compared to healthy volunteers. Psychopharmacology 75:168–371


Rovescalli AC, Brunello N, Franzetti C, Racagni G 1986 Interaction of putative endogenous tryptolines with the hypothalamic serotonergic system and prolactin secretion in adult male rats. Neuroendocrinology, in press


Stanley M, Virgillio S, Gershon S 1982 Tritiated imipramine binding sites are decreased in the frontal cortex of suicides. Science (Wash DC) 216:1337–1339


Wagner A, Åberg-Wistedt A, Åberg M, Ekqvist B, Märtensson B, Montero D 1985 Lower \textsuperscript{3}H-imipramine binding in platelets from untreated depressed patients compared to healthy controls. Psychiatry Res 16:131–139
Whitaker PM, Warsh JJ, Stancer HC, Persad E, Vint CK 1984 Seasonal variation in platelet \textsuperscript{3}H-imipramine binding: comparable values in control and depressed populations. Psychiatry Res 11:127–131

DISCUSSION

	extit{Murphy:} Concentrations of amines in the pineal gland vary during the day/night cycle. In particular, serotonin accumulates in the day-time and melatonin levels are highest at night. So I wonder whether concentrations of 5-methoxytryptoline in the pineal would fluctuate if you measured them at different times over the 24 h cycle. What time did you remove pineals for your studies?

	extit{Langer:} In the studies by Kari (1981) and Kari et al (1983), there is a hint of a circadian rhythm in the levels of 5-methoxytryptoline in the chicken pineal. We obviously could not investigate this in our studies of post-mortem human brain, and even in our rat experiments, when we used pools of 15 pineal glands, the concentrations were too low to embark on a study of possible circadian rhythms (Langer et al 1985). We did a preliminary study in chickens, covering four different times during the day at five-hour intervals, but there was no indication of changes in endogenous levels of 5-methoxytryptoline that could be due to circadian variations. So the question is still open.

	extit{Murphy:} Have there been no studies of pineals obtained during the dark?

	extit{Langer:} Yes. We measured melatonin at different times and got the expected result. In fact, in our 5-methoxytryptoline studies we used deuterated standards for both melatonin and 5-methoxytryptamine, because these two compounds can give rise to artifacts, producing apparent levels of 5-methoxytryptoline that are not due to the genuine presence of this compound.

	extit{Frazer:} I get the impression that you don’t believe 5-methoxytryptoline is the actual endogenous ligand for the imipramine-binding site because of the pronounced effect of temperature on its binding properties. Do you think that perhaps it is simply a ‘lead’ substance? Have you carried out experiments at 37°C to see what happens then to its potency to inhibit \textsuperscript{3}H-imipramine binding?

	extit{Langer:} 5-Methoxytryptoline is an interesting candidate and perhaps a lead compound, but a close analogue may be more biologically relevant. The
decrease in the potency of 5-methoxytryptoline to inhibit \[^3\text{H}\]imipramine binding as the temperature is increased may be related to the thermodynamics of its interaction with the modulatory site for the transporter; in fact if you go from 22°C to 37°C there is an additional loss of potency. At the higher temperature the potency of 5-methoxytryptoline to inhibit \[^3\text{H}\]paroxetine binding becomes identical to the IC\(_{50}\) for inhibiting 5-HT uptake in the same preparation. Nevertheless, at 37°C 5-methoxytryptoline is still more potent than 5-HT at inhibiting \[^3\text{H}\]paroxetine binding. Finally, the issue of potency is perhaps not the main one. There is still the question of the synthetic pathway for 5-methoxytryptoline under normal or pathological conditions, because the enzyme for the closure of the third cycle in the pathway remains to be identified, if it exists.

The question of the compound's endogenous occurrence under normal conditions, particularly in the pineal, is also controversial. We should be very careful about sources of artifacts. Even with mass spectrometry one may not always find 5-methoxytryptoline (Langer et al 1985). It may depend on the sensitivity of the method, and in that sense tandem mass spectrometry offers an advantage. We have detected concentrations up to 2 ng/g, but not as much as 500 ng/g, as reported by Kari (1981) and Kari et al (1983). I feel that in terms of synthesis and storage these concentrations are too low. But if we think in terms of synthesis and immediate release without storage, 1 ng/g may be physiologically relevant. E. Costa (personal communication) finds that after serotonergic denervation with 5,7-dihydroxytryptamine there is a pronounced decrease in the amount of endogenous factor that can be isolated from the brain. Therefore, the modulatory factor may be released as a cotransmitter from the serotonergic terminals. And if it is localized where serotonergic neurotransmission occurs, then the low concentration may still be compatible with a physiological role.

Murphy: We may learn something from thinking analogously about how melatonin acts. Although melatonin is very lipid soluble and does not act directly via a synaptic effect, it is a very active hormonal substance and might serve as a model.

Langer: Melatonin has a methoxy group in position 5 and originates from serotonin, but it is entirely inactive even in millimolar concentrations in competing for the binding site labelled with \[^3\text{H}\]imipramine or \[^3\text{H}\]paroxetine, i.e. in competing with the two ligands that would label the modulatory site of the serotonin transporter. There is a sharp structure–activity relationship; when you N-acetylate the molecule you completely lose activity on \[^3\text{H}\]imipramine binding.

Kelly: The exciting experiment would be to take the pineal itself and to show a labelled substrate being converted to 5-methoxytryptoline.

Langer: That has been done: at least two papers show that labelled tryptophan and 5-hydroxytryptophan act as substrates in cultured pineal glands,
producing labelled 5-methoxytryptoline. But this doesn't prove that 5-methoxytryptoline has a biological role. You can generate trace amounts of many compounds if you start with appropriate labelled precursors and follow the radioactivity, but you cannot conclude from such findings that the product is necessarily formed in physiologically relevant amounts in vivo. The storage of 5-methoxytryptoline may not be like that of monoamines and therefore the post-mortem delay before endogenous levels are determined may be important. 5-Methoxytryptoline may be synthesized only immediately before its release and then degraded quickly, and in these properties it may therefore resemble prostaglandins more than monoamines.

_Murphy:_ If that is so, there might be more available for assay in rodent preparations or tissues from humans treated with monoamine oxidase inhibitors.

_Langer:_ Yes. We have been trying to increase endogenous levels not only by monoamine oxidase inhibition but also by precursor administration. For instance, in rats pretreatment experiments are underway with 5-hydroxytryptophan, a precursor that has been reported to be useful in the management of depression in combination with other therapies, but I do not have sufficient results to report at present.

_Fuller:_ We know that imipramine, paroxetine and other serotonin uptake inhibitors that interact with the imipramine-binding site will all produce very striking potentiation of 5-hydroxytryptophan-induced behavioural or endocrine effects in animals. We might therefore get a clue to the existence of an endogenous modulator of that site if we could show that 5-hydroxytryptophan effects varied markedly with time of day or with the physiological state of the animal. This might reflect an upward or downward movement in the concentrations of an endogenous modulator, depending on whether it was an antagonist or an agonist.

_Langer:_ In the suprachiasmatic nucleus, which acts as an internal clock and has a very dense innervation by serotonergic terminals, there is a circadian rhythm in the uptake of 5-HT (Meyer & Quay 1976). There is also a circadian rhythm in the density of imipramine-binding sites in the rat suprachiasmatic nucleus (Wirz-Justice et al 1983). It's possible that those changes reflect concomitant changes in the levels of an endogenous factor that modulates the neuronal uptake of 5-HT. But it does not follow that circadian changes must exist in the behavioural responses to 5-hydroxytryptophan, because the modulatory factor may not be present throughout the brain. If the changes in imipramine binding in the rat suprachiasmatic nucleus reflect $K_D$ changes, one would be inclined to associate them with variations in the concentration of an endogenous factor. However, it is not clear whether the circadian changes are in the $B_{\text{max}}$ or $K_D$ for $[^3\text{H}]$imipramine binding because the suprachiasmatic nucleus is not large enough to obtain a Scatchard plot with enough points determined at different times of day. Circadian variations in imipramine bind-
ing can also be observed in the raphe nucleus (Kraeuchi et al 1986), and in fact the cell bodies appear to be the sites of synthesis of imipramine-recognition sites, which are then transported down the axons (Dawson et al 1985). So it makes sense to consider cell bodies in the raphe as sites where circadian variations could take place.

**Fuller:** It still seems very important to me to ascertain whether the changes are functionally relevant. I suggested using 5-hydroxytryptophan simply as a means of magnifying any functional changes produced by variations in the amount of serotonin in the synaptic cleft and hence in the amount acting at postsynaptic receptors.

**Kelly:** I don't follow Dr Langer's arguments which relate circadian changes in the uptake of 5-HT into nerve terminals of the suprachiasmatic nucleus to neuronal firing in the raphe nucleus. For instance, the rate of protein transport from the raphe would be insufficient to move proteins related to the imipramine-binding sites to the suprachiasmatic nucleus within one day, never mind several times a day. Thus it is difficult to argue that this process is relevant to the circadian rhythm. Several studies have shown that changes occur in neuronal firing over 24 h cycles in slices cut from the suprachiasmatic nucleus, demonstrating that even when disconnected from the raphe nucleus and from other brainstem nuclei these cells can maintain a circadian rhythm (Shibata et al 1982). Obviously, changes on a circadian time-frame in the raphe may be relevant to depression and to the well-documented changes in sleep, but I'm not sure how they could be related to changes in 5-HT in the suprachiasmatic nucleus.

**Langer:** I did not intend to imply a cause-effect relationship between the circadian rhythm in 5-HT uptake and \(^3\text{H}\)imipramine binding in the suprachiasmatic nucleus and the axonal transport of \(^3\text{H}\)imipramine-recognition sites from the raphe nucleus to the nerve terminals. I think these are independent observations.

**Biegon:** In the rat, 5-HT uptake changes during the oestrous cycle. On the afternoon of pro-oestrus hypothalamic serotonin uptake in the female rat is about four times as high as in the morning (Meyer & Quay 1976). This can be functionally important because serotonin inhibits sexual behaviour in the female rat. You would expect specific mechanisms to be recruited to inhibit serotonergic activity until the time when sexual activity should occur, which is the night between pro-oestrus and oestrus. This is not the circadian rhythm observed in males; it is a four-day to five-day cycle in which changes in serotonin uptake are probably subject to hormonal regulation.

**Langer:** All these observations are of considerable interest but the best evidence for the existence of an endogenous modulator of 5-HT uptake would be to find the molecule, determine its structure and demonstrate that it is synthesized in vivo and produces the expected effect. We have evidence compatible with the existence of an endogenous modulator, and this may