isolated synaptosomes in line with their clinical potency. Thus \textit{trans}-flupenthixol had no action on dopamine release but the \textit{cis}-isomer inhibited $K^+$-stimulated release by 50\% at the dose used (Fig. 1b). Further work is required to establish whether the stimulus-induced release of $K^+$ can be extinguished by higher doses of the \textit{cis}-isomer. In parallel, $K^+$-stimulated dopamine synthesis in synaptosomes from the neuroleptic-treated rats was significantly inhibited. This inhibitory action of the drugs is similar to that previously reported by Seeman & Lee (1975), who used striatal slices, preloaded with $[^3]$H)dopamine. In these experiments, Seeman & Lee (1975) were able to show a good correlation between inhibition and clinical effectiveness of a range of neuroleptics. Insufficient experiments on depolarization-induced release have been carried out in the present study to attempt a similar correlation. However, the data we have accumulated on the inhibitory action of neuroleptics on dopamine taken together with our evidence for an inhibitory action of these neuroleptics on that synthesis of dopamine that is triggered by $K^+$ depolarization would lead us to the conclusion that inhibition of recently synthesized dopamine leads to a parallel decrease in its release.


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\textbf{Pharmacological and Physiological Correlates of Variable Receptor Sensitivity}

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It has been suggested by Raff (1976) that states of variable sensitivity of the cell membrane could play a fundamental role in the homoeostatic control of neuronal activity. Fleming (1976) has even attempted a formal definition, a 'law of innervation', of variable sensitivity, namely 'when functional nerve activity is chronically increased or decreased

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(surgically, physiologically, pathologically or pharmacologically) the sensitivity of most distal effectors... is slowly altered in a direction which compensates for the altered neural input. This regulatory phenomenon is well documented with respect to peripheral synapses (adrenergic: Trendelenburg, 1966; cholinergic: Thesleff, 1974). As far as the central nervous system is concerned several authors have critically reviewed the available evidence (Yarbrough & Phillis, 1975; Sharpless, 1975; Fleming, 1976) to include adrenergic and dopaminergic systems. It is the purpose of the present account to focus attention on the central β-adrenergic receptor and to survey the experimental techniques which have been applied to the quest for quantification of variable sensitivity. Paradoxically, it is instructive to consider first those studies carried out on a peripheral structure, the pineal gland. Here, the possibility that endogenous variations in β-receptor sensitivity contribute to physiological responses has been elegantly demonstrated (Romero et al., 1975; for review, see Kebabian et al., 1977). The pineal of the rat, both functionally and anatomically, is a simple experimental neural preparation (Axelrod, 1974; Romero, 1976). Sympathetic nerves whose cell bodies lie in the superior cervical ganglia innervate the gland through well-characterized β-receptors. Stimulation of these receptors, both in vivo and in vitro, leads to a rapid elevation of cyclic AMP concentration followed by the typical physiological response, secretion of the presumptive pineal hormone melatonin. Biosynthetic activity of the pineal is entrained to the light/dark cycle; thus, under the influence of darkness, sympathetic nerves release noradrenaline which stimulates the β-receptors. Before the end of the dark phase these receptors become relatively refractory to further stimulation. At the beginning of the light phase, however, nervous activity ceases and the sensitivity of the β-receptors then increases gradually to reach a maximum coincident with the onset of darkness. It is apparent therefore that this oscillating level of sensitivity occurs rapidly under physiological conditions; it constitutes the drive for an endogenous circadian rhythm of biosynthetic activity, and is the consequence of circadian changes in neuronal function. As described below, the variations in β-receptor sensitivity have been carefully examined by different groups of workers (see e.g. Kebabian et al., 1977). These studies will form the basis of a consideration of likely fluctuations in central β-receptor sensitivity.

In accordance with Fleming's Law of Innervation, the sensitivity of pineal β-receptors may be predictably altered by pharmacological means. For example, depletion of neural noradrenaline by reserpine treatment or by denervation results in enhanced concentrations of cyclic AMP in the pineal gland after stimulation with submaximal concentrations of the specific β-agonist isoproterenol (Deguchi & Axelrod, 1973). A convenient preparation of a 'denervated' pineal is obtained from animals exposed to light for 24h; this treatment inhibits the dark-induced release of endogenous noradrenaline from the sympathetic nerve terminals (Romero & Axelrod, 1975). Conversely, when the denervated or reserpine-treated rats are repeatedly injected with isoproterenol, the superinduction of cyclic AMP is not only prevented but a state of hyposensitivity to further stimulation is produced.

There appear to be multiple regulatory sites within the cell at which pineal sensitivity to β-adrenergic stimulation could be controlled. For example, hypersensitive glands exhibit increases in both cyclic AMP-dependent protein kinase and adenylate cyclase activity (Zatz, 1977), whereas in stimulated glands there is an elevation of cyclic nucleotide phosphodiesterase activity [Minneman (1977) and references therein]. In addition, there is some evidence that Ca²⁺ influences the response to stimulation (Wilkinson, 1978). However, of some relevance to this review are those methods that seek to investigate directly the properties of the β-adrenergic receptor. In the pineal gland as well as the central nervous system there is now evidence implicating changes in the density of membrane β-receptors as a factor contributing to variations in sensitivity to stimulation.

The means to quantify receptor populations awaited the preparation of highly radioactive high-affinity β-adrenergic antagonists. The hopes, disappointments and successes of this story are ably and critically recounted in other review articles (Cuatrecasas 1978).
et al., 1975; Lefkowitz, 1976; Haber & Wrenn, 1976; Levitski, 1976; Wolfe et al., 1977; Maguire et al., 1977). However, the use of one such antagonist, [\( ^3\)H]dihydroalprenolol (Lefkowitz et al., 1975), has demonstrated that the binding sites observed in the rat pineal gland are indistinguishable from the \( \beta \)-receptor-coupled adenylate cyclase (Zatz et al., 1976). Further work with this ligand quickly confirmed that variations in responsiveness of the \( \beta \)-receptors were paralleled by changes in the concentration of dihydroalprenolol-binding sites (Kebabian et al., 1975; Romero et al., 1975). Thus stimulation of the \( \beta \)-receptors results in a refractory state typified by a decrease in binding sites, whereas a hypersensitive state is characterized by an increase in the number of binding sites. Significantly, when the \( \beta \)-receptor population was measured over a 24h period it was found to vary in a circadian manner, inversely related to sympathetic activity. This work has also demonstrated that during these fluctuations the affinity of the receptor remained unchanged and that protein synthesis was not required for the increase in number of binding sites. A further observation should be mentioned at this juncture: a change in the number of receptors cannot be the only factor which affects sensitivity. The point is clearly illustrated by the fact that choler toxin, an agent capable of activating adenylate cyclase by a mechanism independent of the \( \beta \)-receptor, is able to induce more cyclic AMP and more protein kinase activity in hypersensitive than in hyposensitive glands (Zatz, 1977). Thus sensitivity changes may be expressed at multiple cellular sites, though these may not always include variations in the number of binding sites. For example, Kebabian et al. (1977) have been unable to obtain differences of dihydroalprenolol binding in surgically denervated pineals. As pointed out by these authors, the absence of an effect of denervation on receptor binding may reflect a lack of precision of the binding assay, such that small changes in receptor number cannot be detected. However, since the lack of correlation between changes in receptor binding and the magnitude of the responses of the cyclic AMP-generating systems is now well-described in the central nervous system (see below), it is convenient to comment here on the lack of precision and reproducibility of the binding assays. Maguire et al. (1977) have made a laudable attempt to provide certain of those insights, or 'tricks of the trade', not normally committed to publication. Nevertheless, the true genesis of non-reproducible binding constants probably lies in the incorrect usage of graphical data plots, such as binding isotherms and Scatchard analyses. A discussion of these difficulties is beyond the scope of this account and the reader is referred elsewhere for compulsory reading (Cuatrecasas et al., 1975; Chang et al., 1975; Akera & Cheng, 1977). A final thought on this problem is warranted by a recent report which suggests the use of a 'standard' receptor and computer-assisted analysis of bound radioactivity to achieve consistency of results (Rao et al., 1977).

As outlined, the physiological simplicity of the pineal gland has permitted important progress to be made in the study of variable sensitivity of \( \beta \)-receptors. This discussion will now be extended to include corresponding experimental paradigms employed in the central nervous system and to a consideration of the similar experimental results which have been obtained there.

The most formidable problem to be encountered in the central nervous system, at least with respect to sensitivity changes, is the sheer anatomical and cellular complexity. In the pineal gland the changes are obviously localized to the post-synaptic membrane. The brain, apparently, possesses \( \beta \)-receptors which are associated with both neuronal and non-neuronal elements (see Wolfe et al., 1977, for discussion). In addition, even those subcellular fractions commonly thought to be synaptosomal are probably contaminated with glial membranes (Henn et al., 1976). Therefore the conclusions reached from the experiments which are about to be described must be tempered with the knowledge that the precise cellular location of the receptors is largely unknown, though at least one group of workers has carefully purified a glial fraction from bovine brain and demonstrated the presence of specific dopamine receptors (Henn et al., 1977). If these problems were not enough, a further consequence of the cellular intricacy of the brain is that treatments used to produce changes in receptor sensitivity are inevitably non-specific. For example, injection of 6-hydroxydopamine (3,4,6-trihydroxyphenethyl-
amine) directly into brain tissue is often used to induce hypersensitive β-receptors (see, for example, Wolfe et al., 1977). Clearly, this approach affects many structures and cell types. Similarly, injection of the whole animal with amphetamine is able to induce hyposensitivity in brain β-receptors. On the other hand, just as for the pineal, it is possible to selectively denervate various structures. Unilateral lesions of the medial forebrain bundle, for example, generate a hypersensitive response to noradrenaline (in terms of cyclic AMP accumulation) in the ipsilateral cortex (Dismukes et al., 1975). The pharmacological manipulation of receptor sensitivity has been largely confined to a study of the changes observed in the adenylate cyclase–cyclic AMP system. Thorough accounts of this work are available elsewhere (Dismukes & Daly, 1976; Baudry et al., 1976; Wolfe et al., 1977). Briefly, the response of the cyclic AMP-generating system obeys Fleming's Law of Innervation. More recently, as demonstrated in the pineal gland, evidence has accumulated which implicates variations in receptor populations in the variable sensitivity of β-receptors. The impetus for this work arose from the application of β-receptor-binding techniques to studies in brain tissue (Alexander et al., 1975; Nahorski, 1976; Bylund & Snyder, 1976; Sporn & Molinoff, 1976). All the evidence suggests that the ligand-binding sites in the brain tissue are identical with the β-adrenergic receptor. It is instructive at this point to take a look at work which has attempted to correlate changes in sensitivity of the cyclic AMP system with alterations of receptor binding in the same preparation. Nahorski (1977) using chick brain and Sporn et al. (1976, 1977) in the rat have demonstrated an increase in sensitivity of the β-receptor-mediated cyclic AMP-generating system in cortex after intraventricular injection of 6-hydroxydopamine. In both instances, the change in sensitivity was fairly slow to appear (96 and 24h for chick and rat respectively) and, at least in rat, reached a maximum at 8 days. Nahorski (1977) further investigated the effects of both reserpine and chronic isoproterenol treatment. Reserpine induces a hypersensitive response within 12h, whereas isoproterenol-induced hyposensitivity was fully developed in 3–6h. In all cases where receptor density was determined the observed changes were in the same direction (e.g. an increase in number in the hypersensitive state), but were clearly of a different magnitude. For example, in the rat (Sporn et al., 1977) the increase in β-receptor density was 50%, whereas maximal levels of cyclic AMP accumulation were double those in control animals. Further work by others (Skolnick & Daly, 1977; Skolnick et al., 1978) in two different rat strains has confirmed that the increase in number of ligand-binding sites does not correlate with the magnitude of response of cyclic AMP. Thus the new steady-state level of receptors after pharmacological disruption of normal neural input appears to be only one of several components which contribute to altered sensitivity to stimulation. This conclusion is in accord with that reached from similar work on the pineal gland. However, it is of note that the changes observed by Nahorski (1977), after either reserpine or isoproterenol treatment, were observable in a matter of hours, a time scale very similar to those which occur endogenously in the pineal gland. It appears reasonable to conclude therefore, that brain β-receptors may also exhibit variable sensitivity as a consequence of physiological processes and that this involvement could be detected by ligand-binding techniques. That is to say, the adaptive mechanism which culminates in a reversible alteration in sensitivity can be regarded as a form of synaptic ‘memory’ and as such may be localized and quantified. Thus the receptor-binding method could provide an invaluable complementary tool to the well-tried techniques of amine concentration, turnover and quantitative microfluorimetry studies (Lichtensteiger & Keller, 1974; Selmanoff et al., 1976; Lofstrom et al., 1976). Further, we can expect the utility of other refinements, such as labelled irreversible ligands, antibodies to the β-receptor (Wrenn & Haber, 1976) and fluorescent-labelled ligands (Atlas & Levitski, 1977).

The final section of this review will briefly consider those experimental preparations which attempt to interpret neuroendocrine processes in terms of variable receptor sensitivity and in addition those which seek to explore the molecular basis of affective disorders. Little information is available at the present time to implicate endocrine changes with alterations in central receptor sensitivity. In the periphery,
however, adrenalectomy significantly elevates the number of β-receptors in the liver (Wolfe et al., 1976), oestrogens induce marked changes in α- but not β-receptors in the uterus (Roberts et al., 1977), and Lefkowitz and his co-workers have demonstrated a thyroid-dependent modulation of β-receptor number in the myocardium (Williams et al., 1977). The brain is a well-described target organ for the action of oestradiol and this steroid is known to control the cyclicity of the reproductive process. The laboratory rat, therefore, provides an excellent experimental preparation with which to study the effects of oestrogen on brain β-receptors. Preliminary work in my laboratory (Herdon et al., 1978; H. Herdon, M. Wilkinson & C. A. Wilson, unpublished observations) indicates that oestrogen-mediated gonadotropin release does affect receptor numbers in brain. This may play an important causative role in the emotional lability associated with the human menstrual cycle, since it has been suggested that one underlying mechanism in affective illness could involve receptor sensitivity changes (Bunney et al., 1977). Thus cyclic variations in blood sex-hormone concentrations may affect the sensitivity of central β-receptors.

The study of receptor mechanisms underlying the pathophysiology of affective illness has been explored in terms of the mode of action of anti-depressant drugs (Banerjee et al., 1977) and the anti-depressant effect of electroconvulsive therapy (Grahame-Smith et al., 1978). Banerjee et al. (1977) have demonstrated that anti-depressants, such as iprindole and desipramine, lower the numbers of β-receptors in rat brain. In contrast, the animal experiments of Grahame-Smith et al. (1978) suggest that electroconvulsive therapy increases the functional activity of brain amines.

In conclusion, the regulation of β-receptor number by brain homeostatic mechanisms may prove to be an important physiological process and one which can now be readily quantified. It should be remembered, however, that the modification of receptor sensitivity is expressed at multiple cellular sites quite distinct from the membrane receptor. These other sites, for example cyclic nucleotide phosphodiesterase and adenylate cyclase, may ultimately require a consideration of purine nucleotides (Maguire et al., 1977), alterations in ion distribution (Hertz, 1977), protein phosphorylation (Greengard, 1978) and even, as yet unknown, neuromodulators (Florey, 1967; Torda, 1977).

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Possible Roles for Purine Compounds in Neuronal Adaptation

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Within the last 10 years, attention has been increasingly focused on the role in neuronal function of nucleotides other than cyclic AMP. In this overview it is intended to summarize some of the factors indicating a role for purine derivatives in neuronal adaptation at the cellular level, and to present a hypothesis that these compounds may also modulate neuronal function by altering the pharmacological characteristics of neurotransmitter receptors.

Adenine derivatives

Interest in adenine derivatives blossomed when Sattin & Rall (1970) described the elevation of cyclic AMP concentration in slices of guinea-pig cerebral cortex which resulted from the application of adenosine. It was also noted that adenosine would...