Genetic and pharmacological models of cholinergic supersensitivity and affective disorders

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Summary. Increased muscarinic sensitivity has been associated with altered hormonal states (hypothyroidism and hyperadrenocorticism), chronic administration of muscarinic antagonists or antidepressants with muscarinic actions, selective breeding for anticholinesterase sensitivity, and certain inbred strains of rats and mice. Thus, both genetic and environmental factors may influence muscarinic receptor sensitivity. The reasonably detailed studies on the selectively-bred rats have revealed that the Flinders Sensitive Line (FSL) rats weigh less, are less active, are more sensitive to muscarinic agonists and to stressors, and have higher concentrations of hippocampal and striatal muscarinic receptors than 'normal', or the selectively-bred, Flinders Resistant Line (FRL) rats. Thus, there are a number of parallels between FSL rats and depressed humans. The FSL rats may be the first animal model of depression to mimic the actual trait of depression, and not just the state.
Key words. Acetylcholine; supersensitivity; depression; Flinders sensitive and resistant lines of rats; animal model; genetic selection; DFP.

Introduction

Following the original proposal of Janowsky and colleagues[29] that depression in humans may be associated with an overactive cholinergic system, the progress in understanding cholinergic mechanisms in affective disorders has been gradual. Recently, there has been a renewed interest in this area because of the following observations: increased sensitivity of affective disorder patients to cholinergic agonists[31, 80, 81], increased muscarinic receptor binding in affective disorder patients and their ill relatives[55], cholinergically mediated rapid eye movement (REM) sleep abnormalities in depressed patients[85, 86] and increased cholinergic sensitivity in antidepressant withdrawal phenomena[11, 12]. Several recent reviews have highlighted the evidence from human studies[11, 12, 32, 33] and our purpose here will be to summarize these results as a background to the animal studies that will be discussed in greater detail.

There have been an extensive number of animal models developed to mimic some aspects of human affective disorders[34, 40, 52, 72, 96]. However, as with the majority of research on human subjects, most of this work focused upon the monoamines (primarily norepinephrine and serotonin) as the mediating mechanisms underlying the behavioral states produced in these animal models[40, 72]. Recently, interest has turned to the increased cholinergic sensitivity that can be induced by chronic antagonist drug treatment[42], or by selective breeding for anticholinesterase sensitivity[62, 69], as potential models of the cholinergic supersensitivity reported to exist in at least some human depressives. In this review we will examine these animal studies and illustrate how the findings are consistent with the hypothesis of supersensitive cholinergic mechanisms in affective disorders.

Human studies

A number of studies have confirmed earlier observations[30] regarding the behavioral suppressing effects of physostigmine[31, 33]. Similar observations have been reported following the administration of the muscarinic agonist, arecoline[58, 79, 81]. Other data suggest that patients with a history of depression may be more sensitive to the REM sleep-inducing[85, 86], neuroendocrine[1, 79] and behavioral[80, 81] effects of muscarinic agonists. However, there is also at least one report which failed to find a significantly greater behavioral response to arecoline in depressed patients[59].

There is some evidence that the increased cholinergic sensitivity may be related to a common neuroendocrine abnormality in affective disorders – the failure to suppress adrenocorticotropic hormone (ACTH) and cortisol following administration of dexamethasone. Carroll et al.[7] demonstrated that physostigmine administration reversed dexamethasone-induced cortisol suppression and Janowsky et al.[33] have reported partial confirmation of these observations. It is also well known that physostigmine administration leads to increased serum concentrations of ACTH, beta endorphin and cortisol[9, 31, 81]. Since dexamethasone-induced suppression of cortisol recurs with remission of depression or after discontinuation of physostigmine treatment, these data support the hypothesis that cholinergic supersensitivity may exist only during the state of depression (i.e., a state marker). Further studies are necessary to resolve the issue of whether the cholinergic supersensitivity model is more suitable for the trait and/or state of affective disorders.

There have been only a few studies of muscarinic acetylcholine receptors (mAChR) in patients with affective disorders. An early report suggested that mAChRs were elevated in individuals who had committed suicide[53]. However, a more recent report failed to demonstrate any differences in mAChR binding between normals and suicides[35]. There are several problems with these studies which preclude any definite statements at this stage. Firstly, there were very little clinical data, so it was not certain how many of the suicides were in fact suffering from affective disorders. Secondly, the cerebral cortex was selected for receptor binding analyses and data about mAChR concentrations in other brain regions are not available. It is possible that the lack of a mAChR change in the cortex of suicides may simply reflect the lack of cognitive differences between suicides and controls. Binding data on mAChR in limbic or other brain regions in suicides and/or affective disorder patients could be illuminating.

A report suggesting that elevated mAChR on cultured fibroblasts may be a biological marker in bipolar affective disorder patients[55] has engendered a considerable amount of interest and controversy. At a symposium on the topic, held at a meeting of American College of Neuropsychopharmacology in December 1984, several groups reported their inability to replicate the original findings. There is now considerable doubt about whether mAChR on fibroblasts are functionally relevant[37]. Certainly, more work on mAChR binding in affective disorder patients is necessary before we can determine whether the cholinergic model is supported.

In sum, several reports are consistent with a cholinergic supersensitivity model of depression in humans although some negative reports also exist. Because of the likelihood of heterogeneous populations of human depressives, the human

literature may be confounded. By studying animals with more uniform genetic backgrounds, additional evidence for the involvement of cholinergic mechanisms in depressive behavioral states has been gathered. We now devote out attention to these animal studies.

Genetic models

Virtually all of the recent reviews of animal models of depression have neglected the genetic component, even though it has long been recognized that genetic factors contribute to the pathogenesis of affective disorders [1, 23]. In this section we will consider two series of studies in rats which have some relevance to depression: The Roman High-Avoidance (RHA) and Roman Low-Avoidance (RLA) lines of rats selectively bred to differ in their ability to acquire an active avoidance response [5] and the Flinders Sensitive Lines (FSL) and Flinders Resistant Lines (FRL) of rats selectively bred to differ in their sensitivity to the anticholinesterase compound, diisopropyl fluorophosphate [69, 82]. Before reviewing the literature on these rats, however, we will devote some attention to studies on inbred mouse strains which have explored the relationship between cholinergic mechanisms and behavioral and physiological variables.

a) Inbred mouse strains

Although none of the studies to be considered in this section specifically discusses how the findings may be related to the cholinergic hypothesis of affective disorders, the reports do illustrate the complexity of the relationships between neurochemical and behavioral variables. The reader is referred to the excellent review by Ingram and Corfman [27] for a discussion of other neurobiological differences among inbred mouse strains.

In one of the earliest reports, van Abeelen and colleagues noted a differential response to cholinergic drugs in inbred mouse strains [91 – 93]. The C57BL/6 strain, which had high initial levels of exploration, exhibited decreases after scopolamine, while the DBA/2 strain, which had low initial levels of exploration, exhibited increases [91]. The D2B6F$_1$ strain, a genetic cross between the two inbred strains, had initial levels of exploration similar to the C57BL/6 strain and exhibited smaller decreases after scopolamine. Other experiments indicated that the strain differences were centrally mediated [92] and that the hippocampal cholinergic neurons were involved [93]. Other workers also noted strain-dependent effects of scopolamine in mice [2, 60].

More recent studies have concentrated on the strain-dependent effects of direct and indirect cholinergic agonists. Thus, there have been reports of differences among inbred mouse strains for the muscarinic agonist, oxotremorine [43], for the nicotinic agonist, nicotine [44, 46, 54], and for the anticholinesterase agent diisopropyl fluorophosphate (DFP) [87]. In general, the C57BL mice were more affected by the hypothermia-inducing effect of both oxotremorine and DFP than were DBA mice, while the C3H mice were least sensitive to oxotremorine and DFP [43, 87]. On the other hand, C3H mice were most sensitive to the stimulant effects of nicotine, while C57BL and DBA mice were most sensitive to the depressant effects [44, 54]. A valuable feature of these studies has been the measurement of cholinergic receptor binding and acetylcholinesterase (AChE) activity in addition to the pharmacological responses to the drugs. These data permitted the investigators to conclude that the genotype-dependent drug effects could not be easily explained by strain differences in either AChE activity or receptor binding [43, 87]. They suggested "that the genetic influences on response to oxotremorine are due to some other factor such as a genetic influence on receptor coupling processes" (Smolen et al. [87], p. 624).

On the basis of these studies the C57BL mice exhibit the greatest sensitivity to oxotremorine, a muscarinic agonist; therefore, they are similar to depressed humans and further work which is orientated towards the cholinergic hypothesis of depression may prove illuminating.

Another series of reports has focused on presynaptic cholinergic processes and behavior. An early report suggested that the capacity for long-term memory is greater in the BALB/C strain than in the C57BL/6 or C57BR strains [28]. These behavioral differences appeared to be correlated with differences in choline acetyltransferase (CAT) activity, the BALB/C mice having greater values. This study also reported that the improvement in memory following hippocampal stimulation was strain-dependent [28]. However, in a more recent study, radial maze performance was examined in these same three strains [77]. The C57BR performed most efficiently, followed by the BALB/C and C57BL. It was also reported that there were no significant differences in CAT activity between the strains, although the trends were similar to the earlier report [28]. At any rate there was no correlation between behavioral performance and CAT activity in these strains. Thus there is some discrepancy between the studies. A factor which may have been overlooked in these studies is the possibility that some of the differences among the mouse strains may be related to differences in postsynaptic cholinergic mechanisms, e.g. mAChR binding.

Still another possibility that needs to be considered is that the behavioral differences among the mouse strains may be related to differences in other neurotransmitter systems [27]. For example, because it is well known that the cholinergic system interacts with the dopaminergic system in the modulation of locomotor activity, it is possible that the differential behavioral effects of scopolamine on exploratory activity in mouse strains [91] may be related to differences in dopaminergic function [19]. The possibility of opiate-cholinergic interactions could also be examined [20].

This brief and selective summary has not been exhaustive but should be sufficient to indicate that there definitely are genotype-dependent cholinergic responses in mice. There are also genotype-dependent behavioral differences in mice. However, these behavioral and pharmacological differences cannot be easily accounted for by either presynaptic (CAT activity) or postsynaptic (mAChR binding) cholinergic mechanisms. Further behavioral and biochemical comparisons of the C57BL (cholinergic-sensitive) and DBA (cholinergic-resistant) strains of mice could be fruitful.

b) Roman lines

Since their original development by Giorgio Bignami in Rome, the RHA and RLA lines of rats have been maintained in separate colonies in Switzerland, England and Canada, among other places. In this brief section we wish to highlight some of the findings which are relevant to cholinergic supersensitivity and affective disorders. Driscoll and Bättig [15] recently reviewed much of the data available on the two lines and concluded that the main behavioral differences between them were related to emotional behavior and reactions to stress, variables which are known to be influenced by the cholinergic system [32].

A number of studies have reported differences in sensitivity to cholinergic agonists in the two lines. The RLA line, which is regarded to be the more emotional of the two lines [15], was more sensitive to the behavioral effects of oxotremorine and physostigmine [6, 48, 49]. However, these differences in drug sensitivity could not be attributed to differences in mAChR, there being no differences in receptor concentrations in the several brain regions studied [63]. It is possible that the differ-

ences in sensitivity to cholinergic agonists between the RLA and RHA lines may be related to the reported differences in benzodiazepine receptors[22] and/or to differences in serotonin turnover[14], as both of these systems may interact with the cholinergic system[32, 33, 73]. Alternatively, they may be related to genetic influences on receptor coupling, as suggested for the inbred mice earlier.

However, a recent study reported that the RLA rats had higher rates of spontaneous alternation and more rapid acquisition of a delayed reinforced alternation test than the RHA rats[26]. This study is consistent with other reports that the RLA rats perform better on some learning tasks than the RHA rats[15] and that their poor performance in the active avoidance task is a consequence of motor performance factors, not of learning ability. It is pertinent to point out here that the behavioral differences between the RLA and RHA rats can be related to postulated differences in the cholinergic system: a overactive cholinergic system in the RLA rats would be expected to lead to poorer active avoidance, better passive avoidance performance and higher rates of spontaneous alternation[15, 26]. A recent, interesting finding in this regard has been that RLA rats show higher levels of CAT activity in striatum and hippocampus than do RHA rats[17]. Thus, the RLA strain of rats exhibits behaviors and sensitivity to cholinergic drugs which indicate that its cholinergic system is overactive. Consequently, these rats may provide a useful animal model for human affective disorder patients, who are also more sensitive to muscarinic agonists as indicated above. However, the concept of anxiety (emotionality) must also be considered in this context. It could be argued, for example, that the RLA rats are genetically more anxious because of lower benzodiazepine receptors and it is this state which predisposes them to becoming behaviorally depressed or immobile. A potential direction for further research is interbreeding studies between the lines to assess genetic relatedness of the various behavioral and neurochemical differences.

c) Flinders lines

In the mid 1970's Overstreet and Russell initiated a selective breeding project in Australia in order to study the mechanisms underlying sensitivity and resistance to anticholinesterase compounds[83]. It was known at the time that insects frequently developed resistance to organophosphate anticholinesterase compounds as a consequence of changes in the enzyme, AChE. On the other hand, it was also well known that tolerance development to anticholinesterase compounds was readily observed and occurred in the absence of marked changes in AChE. In order to pursue this question, selective breeding of rats which were either highly sensitive or relatively resistant to the behavioral and physiological effects of DFP were conducted[62]. It soon became apparent that only unidirectional selection was occurring: the Flinders sensitive line (FSL) of rats was more sensitive than randomly bred Sprague-Dawley rats to DFP but the Flinders resistant line (FRL) was not more resistant[62, 82]. It is important for the following discussion to keep this point in mind.

In the course of developing the FSL and FRL rats, a sex difference in sensitivity to DFP was discovered, with females being less affected than males at the standard dosing regimen[61]. This sex difference complicated the breeding program and necessitated the testing of both males and females at each generation. Nevertheless, it became evident that both males and females of the FSL rats were more sensitive to DFP than their like-sexed counterparts of the FRL[62, 82]. Recently, we carried out an interbreeding study in which we compared the F_1 and F_2 progeny with the parental lines. This study suggested that the sensitivity to DFP was related to one or more recessive genes in the males, but the genetic regulation was more complex in the females[66].

Because several experiments indicated the lack of involvement of AChE in the sensitivity of FSL rats[62, 84], we turned our attention to other possible mechanisms. Early psychopharmacological studies indicated that the FSL rats were more sensitive to the behavioral suppressing effects of muscarinic agonists and, under certain conditions, were less sensitive to the locomotor stimulatory effects of muscarinic antagonists[64]. These results suggested that the FSL rats had increased mAChR sensitivity. Subsequent studies have confirmed the increased sensitivity of the FSL rats to muscarinic agonists, using a range of behavioral and physiological indices[8, 57, 69, 70, 94]. These findings are summarized in table 1.

It will be remembered that human depressed patients also exhibited increased behavioral and hormonal responses to muscarinic agonists[31, 80, 81]. Thus, there is a parallel between the FSL rats and human depressives. A study of sleep parameters in FSL and FRL rats under baseline and drug challenge conditions has demonstrated that FSL rats exhibit REM sleep abnormalities similar to those seen in human depressives (e.g. Sitaram et al.[85, 86] and Shiromani et al. in preparation).

It is abundantly clear, therefore, that FSL rats exhibit a cholinergic supersensitivity (table 2). However, as shown in table 1, there are also some differences between the FSL and FRL rats in their responses to drugs acting on other neurotransmitter systems. The stereotypy-inducing effects of apomorphine, a dopamine agonist, are reduced in the FSL rats[8], while the locomotor depressive effects of m-chlorophenylpiperazine, a serotonin agonist, are increased[94]. In contrast, the hypothermia induced by both agents was significantly greater in the FSL rats[8, 94]. At first glance, these findings may seem confusing; however, it is possible to relate them to the way in which the three systems interact in regulating the two functions. The serotonergic and cholinergic systems are parallel systems in the regulation of both locomotor activity and temperature, i.e., stimulation of either leads to behavioral depression and hypothermia. By contrast, the dopaminergic system is an opposing system in the regulation of locomotor activity, i.e., stimulation leads to

Table 1. Altered drug sensitivity in FSL and FRL rats

Agonist drug	Receptor	Measure	Direction of effect	References
Pilocarpine	mACh	Locomotor activity	FSL > FRL	64
Oxotremorine	mACh	Temperature	FSL > FRL	57
Arecoline	mACh	Operant response	FSL > FRL	69
Arecoline	mACh	Corticosterone	FSL > FRL	70
Apomorphine	DA	Temperature	FSL > FRL	8
Apomorphine	DA	Stereotypy	FSL < FRL	8
m-chlorophenyl piperazine	5-HT	Locomotor activity	FSL > FRL	94
m-chlorophenyl piperazine	5-HT	Temperature	FSL > FRL	94
m-chlorophenyl piperazine	5-HT	Operant response	FSL > FRL	94

Table 2. Possible parallels between depressed humans and FSL rats

1)	Lower body weight
2)	Lower general activity
3)	Enhanced responses to cholinergic agonists
4)	Elevated muscarinic receptors
5)	Enhanced responses to stressors

behavioral activation, and a parallel system in the regulation of temperature. It is noteworthy that the RLA line of rats, which are more sensitive to muscarinic agonists, are also more sensitive to the hypothermic effects of apomorphine [16] and less sensitive to its stereotypy-inducing effects [18].

A potentially important observation about the FSL rats is that the cholinergic supersensitivity in these animals may be mediated by different mechanisms in different brain regions. In rats that were shipped to the UCLA Department of Pharmacology, a tail vein injection of deuterium-labeled choline was administered 1 min before sacrifice by microwave fixation [67]. This procedure permitted the simultaneous assessment of both endogenous and labelled ACh, as well as its precursor, choline. There were no differences in endogenous choline or ACh, or labeled choline. The main finding was a regionally specific increase in ACh synthesis in the cerebral cortex of the FSL rats. In parallel studies of mAChR binding in the lines of rats, significant increases in the concentrations of mAChRs in the striatum and hippocampus of FSL rats were detected, but there were no differences in the cerebral cortex [67]. These data provide evidence for a presynaptic cholinergic overactivity in the cerebral cortex (increased ACh synthesis) and a postsynaptic cholinergic supersensitivity in the striatum and hippocampus (increased mAChRs). These findings may be relevant to the lack of mAChR differences between suicides and normals [35]; i.e., the cerebral cortex may have been an inappropriate region to examine. As indicated previously, data on mAChR in limbic regions could be very worthwhile.

Because the cholinergic system is involved in virtually all behavioral and physiological variables, the established differences in cholinergic function between the lines [67] may result in some behavioral and/or physiological differences. Indeed, a number of baseline differences between the FSL and FRL rats have been observed, including decreased body weight and locomotor activity [57, 69, 82]. Both of the latter changes are well documented to occur in humans who become depressed, but less information is known about these variables in euthymic individuals with a history of affective disorders. Interestingly, a recent report has established that euthymic patients do indeed show a reduced activity compared to control subjects [97]. This reduction was most apparent during the daylight hours, the active portion of the circadian cycle, which parallels our recent finding that the FSL rats were less active than the FRL rats during the dark portion of the circadian cycle (Overstreet et al., unpublished observations).

On avoidance tasks the FSL and FRL rats exhibit differences which suggest that they may be comparable to the RLA and RHA lines, respectively. Although it is difficult to compare across laboratories using different criteria for behavior tasks, the following observations do seem pertinent. The FSL and RLA (by definition) perform poorly on an active avoidance task [15, 68]. In contrast, the FSL have a significantly better memory on a passive avoidance task [69] and the RLA rats perform better than the RHA rats on similar tasks (Classen, Fuchs, unpublished observations). The studies of active avoidance performance of the FSL rats have not been as extensive, so direct comparisons of the two different lines cannot be made because of differences in experimental design. It is not known, for example, if the performance of the FSL rats is as poor as is commonly reported for RLA rats. It could be worthwhile to compare the two groups of rats under identical conditions. As discussed earlier, the poor performance on active avoidance is entirely consistent with the observed cholinergic supersensitivity in the FSL and RLA rats.

One of the problems that these particular behavioral data present is the apparent lack of parallels with depressed humans. If one considers only the active avoidance data, then a good parallel exists, with the FSL and RLA rats being impaired and most studies suggesting that memory processes, when altered, are impaired in depressive disorders [50]. However, when the performance of the rats on other tasks is considered, the FSL and RLA rats appear to have improved memories and this result is at variance with the human studies. An important consideration here is the recognition that the relationship between cholinergic stimulation and memory processes in animals and humans is not monotonic. Improvement in memory is generally found after only intermediate concentrations of cholinergic agonists [65], both higher and lower doses being ineffective. Such data suggest that memory processes may be impaired in individuals whose cholinergic system is overstimulated, as might well be the case in human depressives. It could be particularly valuable to conduct studies on euthymic patients with a history of depression to assess their memory processes; the prediction is that they would have better memories because of an increased cholinergic sensitivity that is not so high as to be considered pathological.

Another point for consideration is the recent observation that FSL rats exhibit a greater degree of immobility than do FRL rats when exposed to mild stressors [69]. The FSL animals were significantly less active in an open field after exposure to footshock and had longer periods of immobility in the forced swim test, developed originally by Porsolt [72]. These findings have been interpreted in the following manner: the mild stressors (footshock and forced swimming) lead to an activation of the cholinergic system [32]. The released ACh interacts with elevated mAChRs [67] in the FSL rats, thereby resulting in more immobility. There are indications that human depressives may also be more sensitive to stressors [3]. There is a need to test the FSL and FRL responses to other stressors, and for further work with humans, before generalizations should be made.

In summary, there are a number of parallels among behavioral, pharmacological and neurochemical variables in the FSL rats and depressed humans, as indicated in table 2. All of the points listed in table 2 have been observed in the FSL rats, but their occurrence in depressed humans is less clear cut. It is generally well recognized that lower body weight and reduced general activity are associated with depression, and several laboratories have reported enhanced responses to cholinergic agonists. There is less consistency about the reports of elevated muscarinic receptors and enhanced responses to stress. Nevertheless, there is enough consistency between the human and rat data to indicate that further studies of the FSL rats as models for human depression are warranted.

Pharmacological models

One of the earliest pharmacological models of depression was the suppression of locomotor activity induced by reserpine, a drug with widespread actions. This model was useful in promoting research into biogenic amines, but may have distracted attention from cholinergic mechanisms. In fact, there has been very little effort devoted to the possibility of developing an animal model of cholinergic supersensitivity and depression by pharmacological or hormonal means.

Consequently, this section will briefly review literature which suggests that such models may be possible.

a) Muscarinic antagonists

During chronic treatment with muscarinic antagonists such as atropine and scopolamine, several phenomena have been observed, including increased sensitivity to muscarinic agonists and increased concentrations of mAChRs[4, 41, 42, 45, 90, 95]. Because a similar increase in sensitivity to muscarinic agonists has been observed in depressed humans, further studies of rats chronically treated with muscarinic antagonists may prove valuable. As far as we know, there have been very few studies of rats during withdrawal from chronic antagonist treatment. Loullis et al.[39] reported a significantly increased retention on a passive (inhibitory) avoidance task in rats chronically infused with scopolamine via osmotic minipumps and subsequently withdrawn. They interpreted their results as evidence for improved memory; however, their results may also be viewed as due to an increased sensitivity to the footshock stressor in the drug-withdrawn rats. Thus, anticholinergic-withdrawn rats could be regarded as similar to FSL rats[69].

Because cholinergic overactivity has been implicated in the withdrawal effects occurring in humans following termination of antidepressant drugs[11, 12], studies of behavioral measures in rats during withdrawal from chronic antidepressant treatment could also be worthwhile. Although there has been a large number of studies employing chronic antidepressant drugs in animals, very few of these have examined the behavior of the rats during the withdrawal period. Results of behavioral studies could show significant relations, because several investigators have reported significant elevations of mAChR-binding following chronic antidepressant treatment[25, 76].

Another avenue for further research in anticholinergic-withdrawn rats is sleep studies. Preliminary studies indicate that rats withdrawn from chronic scopolamine treatment exhibit an elevated incidence of REM sleep during the withdrawal period[88]. This finding may relate to the increased REM density seen in depressed patients. Sleep studies with these rats are continuing.

Recently we carried out a study in which we compared the selectively-bred FSL and FRL rats with randomly-bred rats chronically treated with saline, scopolamine or amitriptyline, and subsequently withdrawn. As summarized in table 3, the FSL rats exhibited the greatest degree of immobility in the forced swim test and the scopolamine- and amitriptyline-withdrawn rats were also significantly more immobile than the saline-treated FRL rats[71]. Thus, cholinergic supersensitivity induced by either selective breeding or chronic drug treatment led to increased immobility under conditions of stress.

Table 3. Forced swim test in Flinders sensitive line (FSL), Flinders resistant line (FRL), and rats withdrawn from chronic treatment with saline (SAL), scopolamine (SCOP) or amitriptyline (AMI)

Group	Median % time immobile ± SIR
FSL	64.5 ± 6.3[a]
SCOP	54.0 ± 5.4[a, b]
AMI	46.7 ± 4.3[a, b]
FRL	36.7 ± 1.8
SAL	34.0 ± 2.7

Rats were placed individually in a 25 °C water tank for 5 min and the time spent immobile (minimal movement of paws) was recorded. From this a percentage score was calculated. Data from Overstreet et al.[71]. [a] Significantly different from FRL and SAL groups. [b] Significantly different from FSL group.

b) Hormones

Although there is a fairly extensive human literature on the predisposing influences of hypothyroidism, hyperadrenocorticism and oral contraceptives towards depression, there have not been any specific studies on animals that have considered these abnormal hormonal states as models for affective disorders. Consequently, in this section a brief consideration of the influence of these altered hormonal states on cholinergic mechanisms will be given, in order to illustrate the potential usefulness of hormonally-modified animals as models for affective disorders.

In considering the thyroid system, two aspects are relevant. These are the consequences of hypothyroidism or thyroidectomy, and of hyperthyroidism or chronic treatment with thyroid hormones. There is an extensive literature on each of these topics, but our purpose here is only to survey those papers which have reported effects related to the cholinergic system. Interestingly, experiments indicate that both thyroidectomy and chronic thyroxine treatment lead to peripheral cholinergic supersensitivity in rats[21, 24]. However, in the former case, it was a change in the maximal response of the muscle, while in the latter there was a shift in the dose-effect curve to the right. Studies are in progress to determine if altered thyroid states have an influence on central mAChR binding.

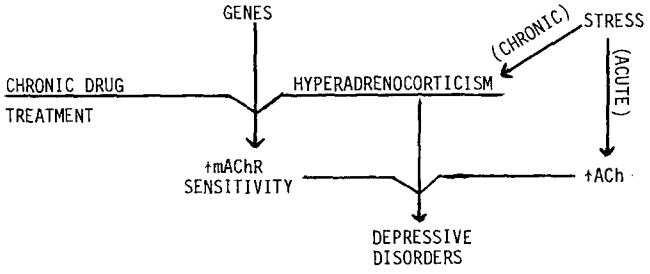
One of the reports on the effects of thyroidectomy has suggested that these might be mediated by an increase in glucocorticoids[24]. In fact, there are several independent reports of increased cholinergic function in rats chronically treated with synthetic glucocorticoids[47, 78, 89]. Some of these have demonstrated a specific regional increase in choline uptake, indicative of an increased turnover of ACh, and others have reported increased receptor sensitivity. Such data are consistent with the hypothesis that hyperadrenocorticism may be a predisposing factor to depression, because the glucocorticoids lead to increased mAChR sensitivity. There have been comparatively few behavioral studies of rats with elevated adrenocortical function, and such studies could be very important in furthering our understanding of depression, particularly that seen in Cushing's syndrome[36].

Yet another hormone that may influence cholinergic mechanisms in animals is the female hormone, estradiol[51]. Among the findings is the observation that the concentrations of hypothalamic mAChR are increased by treating ovariectomized female rats with estradiol[74]. Similar brain regions in male rats appear to be unresponsive to estradiol treatment[13]. A recent paper has suggested that these increased concentrations of mAChR are functionally related to activation of female sexual behavior[75]. These elevated receptors may partially account for the sex differences in oxotremorine-induced hypothermia[56]. Female rats also show more drinking induced by lateral hypothalamic administration of oxotremorine (Netherton, unpublished PhD thesis).

In summary, there are animal studies which indicate that decreased thyroid function, increased adrenocortical function and increased estrogen levels can all lead to increased cholinergic sensitivity. Although each of these hormonal states produces a variety of other changes, it is possible that their predisposing influences on depression could be mediated through cholinergic mechanisms.

Integrative models

The model in the figure is presented as an integrative model of the data summarized above. In the model, genetic factors, hyperadrenocorticism, and chronic drug treatment are considered predisposing factors, and stress is considered a precipitating factor. The evidence for the predisposing factors reviewed above suggested that they may all induce a cholin-



Genes, hormones, drugs, and cholinergic supersensitivity. This model proposes that genetic factors, chronic drug treatment or high adrenocortical status can lead to increased mAChR sensitivity. Upon interacting with supersensitive mAChR, acetylcholine (ACh), whose release may be stimulated by acute stressors, can produce depression. Chronic stress, by leading to increased adrenocortical function, may also produce elevated mAChR, thereby making the individual susceptible to depressive states upon subsequent exposure to acute stressors.

ergic supersensitivity. The latter may explain why stress can precipitate depression in such predisposed individuals: the stress will lead to increased release of ACh[9,32], acting upon supersensitive mAChR, and leading to depression.

Another aspect of this model is the proposal that stressors may be predisposing, as well as precipitating, factors[3]. Chronic stress, by elevating glucocorticoids for long periods, may lead to increases in mAChR sensitivity, thereby predisposing an individual to depressive states when exposed to subsequent stressors (fig.). Why some individuals become depressed during chronic stress and others do not may be related to genetic factors which influence the regulation of mAChR.

A short-coming in the model proposed in the figure is that it is unitary in its mechanism. It is unlikely that cholinergic supersensitivity will provide an adequate explanation for all forms of affective disorders. Although the model is useful in accounting for some factors, it must be remembered that affective disorders have also been associated with abnormalities in other neurotransmitter systems. We propose the model as one way of looking at a subtype of affective disorders. Another possible model is the suggestion that cholinergic supersensitivity, as produced by genetic factors and/or hormonal influences, acts as a predisposing factor toward mood disorders, while the actual mood alterations may come about through subsequent changes in catecholamines. This model thus integrates the cholinergic hypothesis with the well-known catecholamine hypothesis, but differs from the earlier models put forward by one of us[29] in proposing that cholinergic supersensitivity is a predisposing factor.

The integrative model presented in the figure is not dissimilar to that proposed originally by Akiskal and McKinney[1] in regard to the roles played by genetics and stress. In this paper, however, it has been suggested that animal models of one genetic predisposing factor towards depression can be developed. These models, therefore, differ from previous animal models of depression which have emphasized various environmental factors[34,40,52,72,96]. In his critical review of these models, Willner[96] concluded that "it is therefore possible that stress-based models might prove to be valid as models of a state of depression, even if they should turn out to be wide of the mark as models of its aetiology" (p. 11). The genetic models discussed in this review are more likely to be models of the trait of depression and, therefore, relevant to its aetiology. Both the FSL and RLA rats are more sensitive to cholinergic agonists, as are people with a history of affective disorders. It is possible that both presynaptic and postsynaptic cholinergic mechanisms are involved in these animals[17,67]. Further research, which integrates the stress-based models with the genetic models described in this review, could provide even more insights into mechanisms underlying affective disorders.

Concluding comments

The present review has summarized data which provide support for cholinergic supersensitivity as one mechanism underlying *some* forms of affective disorder, and has suggested that animal models of this predisposing state can be developed. The review was not intended to be exhaustive in all aspects. The reader is referred to recent papers by Dilsaver[10] and Lerer[38], who have integrated the human and animal literature on electroconvulsive shock and lithium treatment as they relate to cholinergic mechanisms in affective disorders. The genetic models reviewed in this paper offer great promise in the search for the underlying basis of affective disorders because they mimic the trait of depression, unlike previous animal models which have mimicked the state.

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