Pergamon

Life Sciences, Vol. 55, No.s 25/26, pp. 2171-2178, 1994

0024-3205/94 $6.00 + .00

0024-3205(94)00398-X

IN SEARCH OF THE MECHANISM OF ACTION OF THE NOOTROPICS: NEW INSIGHTS AND POTENTIAL CLINICAL IMPLICATIONS

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Summary

The positive action of nootropics on the memory has up to now primarily been discussed in the context of effects on energy metabolism and cholinergic or glutaminergic neurotransmission. Recent findings have shown that the memory-enhancing effect is steroid-sensitive. Since corticosteroids are potent modulators of gene transcription, it appears possible that the nootropics may exert a modulatory action on protein synthesis. This assumption is supported on the one hand by the fact that the nootropics improve the memory even if they are administered several hours after the learning trial, and on the other hand by the observation that their memory-enhancing effect does not become detectable until 16-24 hours after the treatment and learning trial. Provided the memory-enhancing effect in animal experiments and the therapeutic effect in patients come about by way of the same mode of action, the fact that high levels of corticosteroids suppress the effects of the nootropics could also have clinical implications: in the light of the observation that the majority of Alzheimer patients have elevated steroid levels it could explain why there is always only a small proportion of patients in clinical trials that respond to treatment with nootropics.

Key Words: nootropics, piracetam, oxiracetam, pramiracetam, aniracetam, steroids

The nootropics are a group of pharmacologically active compounds which in some respects occupy a special position in the pharmacology of the central nervous system. They have definitely positive effects on the retention performance of laboratory animals in various experimental situations such as passive and active avoidance (1-5), radial mazes (6,7), place-navigation tasks (8), matching-to-sample tests (9) and object-recognition tests (10). Moreover, they have some positive effects in patients with memory problems (11-13) or other cognitive disturbances (14-16). On the other hand, in all tests belonging to the standard armamentarium of the psychopharmacological laboratory they are practically inert, and in both humans and animals practically free of toxic effects. Opinions about these preparations are highly divergent. For some, the absence of toxicity indicates a lack of any pharmacological action; others see it as pointing to a new therapeutic approach. Depending on the observer's standpoint, either the non-responders in clinical trials testify to the inefficacy of these agents, or the responders bear out their activity.

Despite numerous experimental studies, all efforts to shed light on the mechanism of action of these agents have so far been unavailing. This review gives a brief account of our own contributions in this field and discusses our findings in the general context of the relevant published work. Another, more remote objective of our research was to seek an explanation for the above-mentioned fact that in clinical studies there is always only a limited proportion of the patients that respond well to treatment (see e.g., (17)).

Biochemistry and pharmacology

The available findings on the mode of action of the preparations can be grouped in four categories: (1) effects on energy metabolism; (2) effects on cholinergic mechanisms; (3) effects on excitatory amino-acid-receptor-mediated functions, and (4) steroid sensitivity.

Energy metabolism

The absence of effects in traditional (transmitter-sensitive) psychopharmacological tests caused the search for the underlying mechanism to be shifted primarily in the direction of energy metabolism. For a long time, the increase in adenylate kinase activity induced by piracetam (18) remained the only biochemical effect detected. These findings were then supplemented by observations on increased uptake of 32P into phosphatidyl inositol and phosphatidyl chloride in glial cells and neurons (19) and an increase in glucose utilization under hypoxic conditions and accelerated recovery in the EEG (20). Piercey et al. (21) observed no increase in glucose utilization in response to piracetam, but a reversal of the depression of glucose utilization induced by scopolamine. The absence of psychopharmacological effects in the above sense was then apparently substantiated, insofar as a series of biochemical investigations of effects on transmitters gave negative or contradictory results. (8,22).

Cholinergic effects

The intensive search for some connection with cholinergic mechanisms was evidently prompted by the findings of Deutsch (23), Bartus (24), and Drachman (25), who showed that cholinergic mechanisms were involved in learning and memory processes, and by the reports of a cholinergic lesion in Alzheimer patients (26,27). The discovery that pramiracetam increases high-affinity choline uptake (22) sparked off a whole series of similar investigations with other nootropics (28-31) that produced similar results. Oxiracetam was shown to prevent the decrease in the acetylcholine content of the cortex and hippocampus resulting from cerebral electroshock treatment (32). The observations that piracetam increases the density of the muscarinic cholinergic receptor in the frontal cortex of aged rats (33) also pointed to the involvement of cholinergic effects. When this selection of findings on the effects of piracetam-like substances is viewed against the background of the already available evidence for the involvement of cholinergic mechanisms in learning and memory, the obvious conclusion is that these substances act by way of cholinergic mechanisms.

Upon closer scrutiny of the available results, however, it can be noted that there is hardly a single study in which several piracetam-like nootropics actually displayed similar effects in parallel tests. On the contrary, in comparative studies the various preparations appear to exert very different effects, i.e., where piracetam is active,

oxiracetam is inactive, and vice versa (22,29,30,32). All in all, the evidence so far adduced in favour of a cholinergic effect of the nootropics is fragile; however, given the attractive concordance between Alzheimer pathology, the therapeutic concept, and the theoretical mode of action these contradictions were evidently found to be of negligible importance.

Excitatory amino acids

In the recent publications, a new line of research appears to be blossoming forth. The sudden deluge of literature on a possible common basis of long-term potentiation (LTP) and memory (34,35) has set off a growing trend to associate the effects of nootropics with some interaction or other involving glutamate transmission. Accordingly, it has been shown that oxiracetam can partially antagonize the behavioural disturbance induced by AP 5 (36,37). Moreover, oxiracetam has been found to increase glutamate release in hippocampal slices (38). In an oocyte expression system as well as in hippocampal tissue it has been shown that aniracetam amplifies currents mediated by the AMPA receptors (38,39) The modulation of AMPA receptors by aniracetam was of interest above all in view of the fact that LTP also amplifies receptor-mediated currents. In the meantime, however, doubts have arisen as to the validity of the assumption that the receptor changes induced by LTP and aniracetam are the same (40).

Regardless of how the controversy may ultimately be settled, it seems probable that, for a variety of reasons, the results now available will be of very little help in clarifying the central problem of the mode of action of the nootropics. In the first place, there is still too much uncertainty about the connection between LTP and learning (34). Secondly, considering one single receptor in isolation places various aspects of the action of a substance in a highly artificial light. It is therefore quite possible that the changes observed have nothing whatever to do with the envisaged effect on memory. Thirdly, as yet, no comparative studies with different nootropics have been published. Since Xiao et al. (39) point out that the response-enhancing properties of aniracetam are not common to all nootropics, because other drugs in this class exert either weaker effects or none at all, it is highly improbable that these experiments could help to clarify the mechanisms underlying the memory-improving effects of the nootropics. The similarity of the effects of the piracetam-like nootropics on memory and their very close structural relationship nevertheless strongly suggest that they all improve the memory by way of the same mechanism. There is consequently ample reason to continue searching for a mechanism shared by all the representatives of this class of substances.

The involvement of steroids

Two aspects in particular led us to consider the possibility that steroids might somehow be involved in the effects of the nootropics. One was the possible existence of internal memory-boosting mechanisms, which — usually in conjunction with highly emotional states — can evoke 'flashbulb memories' (41). The other was the observation that autoradiographs taken after the administration of radiolabelled oxiracetam showed practically no activity in the brain (42). The fact that emotional states (43), ACTH (44), and steroids (45) can augment memory storage prompted us to look for indications linking the effects of nootropics to adrenal function. Surprisingly, it turned out that oxiracetam, piracetam, pramiracetam, and aniracetam no longer exerted any memory-enhancing effects in adrenalectomized animals (42,46), although the animals' general learning capacity was not impaired by adrenalectomy. Similarly, pretreatment with

aminoglutethimide (47), which, in effect, produces a chemical blockade of the adrenal cortex, rendered the four piracetam-type nootropics inactive (48). Aminoglutethimide itself had no effects on the retention performance of the mice at the dose used. These findings were the first indication that adrenocortical products played a part in mediating the effects of the piracetam-like nootropics. In a second series of experiments, we studied the influence of a mineralocorticoid antagonist on the memory-enhancing effect of the nootropics. Pretreatment with epoxymexrenon, a potent mineralocorticoid antagonist (49) produced a similar result: the memory-enhancing effect of the nootropics was completely blocked. Again, the steroid blocker per se had no effect on learning capacity (48). This was a first indication that the mineralocorticoid receptors (Type I) were somehow involved in bringing about the nootropic effect.

A further series of experiments then furnished first of all proof that substitution therapy with aldosterone or corticosterone in adrenalectomized animals can restore sensitivity to the effect of the nootropics, and secondly a further indication that the mineralocorticoid receptors play an important part in mediating the effects of nootropics: substitution with corticosterone or aldosterone was ineffective in adrenalectomized animals if at the same time the Type-I receptors were blocked by epoxymexrenon (50). The results of these substitution experiments additionally suggested that higher doses of the hormones produced weaker or no effects. Logically, that posed the question whether elevated plasma concentrations of steroids could likewise suppress the memory-enhancing effects of the nootropics. The experiments carried out to explore this possibility showed that pretreatment with corticosterone or aldosterone not only raised the hormone levels, but also suppressed the memory-improving effects of all the nootropics (5). The parallel control experiments with arecoline, physostigmine, and tacrin (THA) yielded the totally unexpected finding that an increase in the steroid levels likewise suppressed the memory-enhancing effect of these cholinomimetics. This opened up the possibility that any form of pharmacologically induced memory improvement might be blocked by steroids. In the course of another study (51) it was demonstrated that the memory-enhancing effects of the glycine antagonist strychnine, the ACE inhibitor captopril, the Ca antagonist nimodipine, and the NMDA receptor blocker CGP 37849 can, in fact, also be completely suppressed by pretreatment with corticosterone and aldosterone.

An overall evaluation of the findings made with the four nootropics, the three cholinomimetics, and the other four memory-enhancing substances strengthened the hypothesis that every pharmacological improvement of memory is mediated by some steroid-sensitive pathway. Although this conclusion strictly speaking only holds for the passive-avoidance situation tested, initial experiments have already shown that blockade of aldosterone biosynthesis by a specific inhibitor, CGS 20287, suppresses the memory-enhancing effect normally induced by oxiracetam in a social-recognition test in rats. Moreover, an extensive series of control experiments had revealed that the memory-disturbing effects of scopolamine, phenobarbitone, diazepam, and CGP 37849 were not impaired by steroidal pretreatment (51). Particularly interesting results were obtained with the NMDA receptor blocker CGP 37849: the same dose of 3 mg/kg improved retention performance in the step-down passive-avoidance test and impaired it in the step-through passive-avoidance test. Whereas pretreatment with aldosterone or corticosterone completely suppressed the memory-improving effect in the step-down situation, the memory-disturbing effect in the step-through test situation was insensitive to the steroids (51,52). The results accumulated so far do in fact appear to favour the hypothesis that steroids selectively block pharmacologically induced improvements of memory.

Implications for basic research on long term memory

The fact that drugs belonging to such diverse categories as nootropics, cholinomimetics, Ca antagonists, glycine antagonists, and NMDA blockers were all capable of improving retention performance in our series of experiments clearly illustrates that the mechanism underlying an effect on the memory cannot merely be inferred from the generic label a substance may happen to have been given, e.g. 'cholinergic'. The finding that the memory-enhancing effects of all these agents can be blocked by a slight increase in corticosterone or aldosterone levels signifies that somewhere in the chain of events ultimately leading to improvement of retention there are intermediate common links susceptible to the influence of steroids. It was therefore conceivable that our results contained indications pointing to events that intervene after neurotransmission. This possibility served as a beacon for our further studies.

We had already demonstrated that all the tested substances were also active when administered after the learning trial (53). That these drugs still improve memory even if they are given several hours after the learning trial can only be interpreted in terms of some modulatory influence on processes still ongoing for some considerable time after the end of the learning trial. Since steroids are potent modulators of gene transcription (54,55), and the nootropics apparently interact with a process long outlasting the duration of the experiment, it would be unreasonable to reject the hypothesis that the effect of the latter compounds on memory might be brought about by modulation of gene transcription or protein synthesis (53) especially in the light of whole series of experiments showing that protein-synthesis inhibitors can also block the formation of long term memory (56,57). Granting the possibility that a learning experience may lead to the activation of genes, it must still be borne in mind that such processes take time. If the nootropics do, in fact, exert an influence on sequential processes of this nature, it follows that the memory-enhancing effect will only come into evidence after a certain time-lag. Differences between treated and untreated animals would only become manifest from that moment on when memory is dependent on the products of the processes modulated by the nootropics.

In a large series of experiments, we found that the effects of oxiracetam only became measurable about 16-24 hours after the learning trial, regardless of whether the drug was given before it or afterwards; not until then was the retention performance of the treated animals appreciably better than the controls' (58). Since a stable drug effect was still in evidence at all later intervals investigated (up to 16 days), it seems reasonable to suppose that the memory emanated from the same source throughout the entire recorded retention interval of 16 days. Oxiracetam must therefore have reinforced the formation of a substrate necessary for long-term memory. Accordingly, it can therefore be inferred that in these animals "long term memory" comes into operation after about 16-24 hours. The fact that the animals also show some retention after shorter intervals is a clear indication that recollection during these intervals occurs by way of different mechanisms not sensitive to the effects of oxiracetam.

In view of the above mentioned findings pointing to the involvement of protein synthesis in the establishment of long-term memory traces (56,57), there was obviously a need to review the observed dynamics of the long-term memory in the light of the latest findings on the dynamics of protein synthesis. ' These show that neuronal signals can activate immediate early genes (IEG's) within a matter of minutes (59-61), but that hours elapse until the transcription factors produced by IEG's have fully activated the late genes (62). In the case of the NGF gene, for example, the transcription rate only reaches its maximum after about 12 hours. These figures nicely correspond with the above mentioned results showing that the nootropics can improve retention performance if they are administered within up to 12 hours and longer after the

learning process (53,63) Th period during which the memory is susceptible to such modulatory influences could, for instance, thus reflect the presence of the transcription factors triggered off by the learning situation.

No matter whether this whole series of experiments does or does not really open up intriguing new perspectives in the above sense, it should not be forgotten that, despite all the experimental evidence, it is still just a theory. Recent findings concerning membrane-bound steroid receptors in the brain (64,65), including some associated with the GABA/benzodiazepine/chloride-channel complex (64), admit of totally different interpretations of how steroids might modulate the effects of nootropics (or vice versa). The palette of possibilities stretches from total peripheral to exclusively central sites of action, and from modulations of brain compartmentation to modulation of protein phosphorylation. Many of these aspects still remain to be explored experimentally before any conclusions can be drawn about the mode of action.

Clinical Implications

Given the available literature on the clinical effects of the nootropics, there can be no doubt whatever that there are patients with a form of dementia that responds very well to treatment with nootropics (see Introduction). A census across all the published reports would put the proportion of such cases somewhere between 10 and 30%. The response rate applies not only to the nootropics, but also to all other preparations tested in the symptomatic therapy of dementia, such as tacrin, etc. (e.g. (66)). In view of these figures it is obvious that investigations in a population of 6-15 patients can never afford a sound basis for any appraisal of clinical efficacy; the likelihood of there being any responders at all is far too small. Debate over the efficacy or inefficacy of these preparations resting on the results of such inadequate studies is consequently futile, above all while many really relevant problems of clinical research still remain unresolved: e.g., whether patients responding to cholinomimetics also respond to captopril, piracetam, etc., or which special physiological or pathological characteristics separate responders from non-responders. Our experimental findings concerning the association between effects on memory and endogenous steroid levels could provide a first clinically verifiable approach in the right direction. Several studies have already shown that a large percentage of Alzheimer patients have very significantly elevated plasma cortisol levels (see e.g., (67)). Should it prove that treatment with nootropics or cholinomimetics is less effective in patients with very high or very low plasma concentrations of cortisol (or aldosterone), then there might be possibilities of identifying potential responders in advance and improving the therapeutic prospects by treating them with inhibitors of steroidogenesis or steroid antagonists.

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