Pharmacology Biochemistry & Behavior Vol 27, pp 491–495 © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Interactions Between Oxiracetam, Aniracetam and Scopolamine on Behavior and Brain Acetylcholine

GIACOMO SPIGNOLI AND GIANCARLO PEPEU[1]

Department of Preclinical and Clinical Pharmacology, University of Florence, Viale Morgagni 65, 50134 Florence, Italy

Received 26 September 1986

SPIGNOLI, G AND G PEPEU *Interactions between oxiracetam, aniracetam and scopolamine on behavior and brain acetylcholine* PHARMACOL BIOCHEM BEHAV 27(3) 491–495, 1987 —The effect of cognition-enhancing agents oxiracetam and aniracetam on scopolamine-induced amnesia and brain acetylcholine decrease was investigated in the rat Acetylcholine levels were measured by means of a gas-chromatographic method Scopolamine (0 63 mg/kg IP 60 min before training) prevented the acquisition of a passive avoidance conditioned response ("step through" retest 30 min after training) and brought about a 64, 56 and 42% decrease in acetylcholine level in the cortex, hippocampus and striatum respectively Oxiracetam (50 and 100 mg/kg IP) administered 30 min before scopolamine reduced the scopolamine-induced amnesic effect and decrease in acetylcholine level in the cortex and hippocampus, but not in the striatum Lower and higher doses of oxiracetam were ineffective Aniracetam (100 mg/kg PO) also prevented scopolamine-induced amnesia but attenuated acetylcholine decrease in the hippocampus only Aniracetam (300 mg PO) reduced acetylcholine decrease in the hippocampus but did not prevent scopolamine-amnesia In conclusion, oxiracetam and aniracetam exert a stimulatory effect on specific central cholinergic pathways However, a direct relationship between cognition-enhancing properties and cholinergic activation needs further confirmation

Aniracetam Oxiracetam Cognition-enhancing agents Scopolamine Brain acetylcholine Amnesia

ANIRACETAM (1-anisoyl-2-pyrrolidinone) prevents the impairment in learning and memory induced in rodents by hypercapnia, scopolamine, and electroshock inhibition of brain protein synthesis [3] Oxiracetam (4-hydroxy-2-oxopirrolidinoacetamide) improves memory and learning ability in normal animals [20] and in rats whose cognitive functions are impaired by methylazoxymethanol [1] For these reasons oxiracetam and aniracetam can be considered cognition-enhancing or nootropic agents, a class of psychoactive drugs, whose prototype is piracetam [8], selectively improving the higher telencephalic, integrative activities [7] Clinical effects of the cognition-enhancing agents have been also reported [18–19]

Oxiracetam, piracetam [15] and pramiracetam [16] have been shown to stimulate high affinity choline uptake Under some conditions piracetam also decreases acetylcholine [ACh] level in the hippocampus [2,25] Oxiracetam prevents the decrease in brain levels and memory impairment induced by electroshock in the rat [23] These findings indicate that cognition-enhancing agents may act on brain cholinergic mechanisms whose role in cognition and memory has been well documented [4,5]

Scopolamine and all centrally acting antimuscarinic drugs induce a transient disruption of memory in man and animals by blocking postsynaptic muscarinic receptors Their effect can be antagonized by physostigmine [10,14] which increases brain ACh levels It can be therefore assumed that the reversal of the transient disruption of memory by oxiracetam and aniracetam could also be due to an increase in activity of central cholinergic neurons We sought support for this hypothesis by investigating the effect of oxiracetam and aniracetam on scopolamine-induced impairment of a passive avoidance conditioned response and on brain ACh level

METHOD

Animals

The experiments were carried out on male Wistar rats (100–120 g body weight) housed in plastic cages in groups of 5 and given free access to food and water in the home cage A normal day and night cycle was maintained with lights on from 7 a m to 7 p m The rats were allocated at random to the different treatment groups

Passive Avoidance Conditioned Response

The apparatus derived from that described by Jarvik and

[1]Requests for reprints should be addressed to Prof Giancarlo Pepeu, Department of Pharmacology, University of Florence, Viale Morgagni 65, 50134 Florence, Italy

TABLE 1

EFFECT OF OXIRACETAM AND SCOPOLAMINE ON PASSIVE AVOIDANCE CONDITIONED RESPONSE

Treatment	Dose mg/kg IP	N Rats	Retest Latencies (sec) mean ± S E
Saline	—	16	109 6 ± 6 3
Oxiracetam	50	7	108 4 ± 9 2
Oxiracetam	100	8	107 5 ± 8 4
Scopolamine	0 63	15	23 3 ± 3 2*
Oxiracetam	10	6	21 7 ± 4 3
+ scopolamine	0 63		
Oxiracetam	50	6	118 3 ± 1 7‡
+ scopolamine	0 63		
Oxiracetam	100	7	69 7 ± 4 8†
+ scopolamine	0 63		
Oxiracetam	300	8	51 3 ± 5 6
+ scopolamine	0 63		

Significantly different from saline *$p<0$ 001 Significantly different from scopolamine alone †$p<0$ 05, ‡$p<0$ 001

TABLE 2

EFFECT OF ANIRACETAM AND SCOPOLAMINE ON PASSIVE AVOIDANCE CONDITIONED RESPONSE

Treatment	Dose mg/kg OS	N Rats	Retest Latencies (sec) mean ± S E
Saline	—	16	109 6 ± 6 3
Aniracetam	100	7	103 9 ± 9 8
Scopolamine	0 63	15	23 3 ± 3 2*
Aniracetam	50	8	28 7 ± 2 7
+ scopolamine	0 63		
Aniracetam	100	6	98 0 ± 6 1†
+ scopolamine	0 63		
Aniracetam	300	6	23 2 ± 2 1
+ scopolamine	0 63		

Significantly different from saline *$p<0$ 001 Significantly different from scopolamine alone †$p<0$ 001

Kopp [11] consisted of two compartments with grid floors which could be electrified separately The first compartment was a 25×25×33 cm Plexiglas box illuminated by a 100 W lamp suspended 50 cm above the box A guillotine door connected the first with the second 50×25×33 cm dark compartment whose walls were painted black

Thirty min after the treatment with oxiracetam (IP) or aniracetam (PO) the rats were injected with scopolamine 0 63 mg/kg and after 60 min they were trained in a one trial passive avoidance task The retest was carried out 30 min after training The rat was placed in the illuminated Plexiglas box After 1 min the guillotine door was opened and the latency between the door opening and the entrance into the dark box was measured (I latency time) with a stop watch When the rat walked into the dark box it received a foot shock by means of a train of impulses (5 Hz, 20 msec, 1 5 mA for 5 sec) delivered by an electronic stimulator The trial was terminated when the rat ran back into the illuminated compartment, from which it was then removed After 30 min the rat was placed again in the illuminated compartment and the time spent without walking into the dark compartment was measured (II latency time) The maximum time allowed for the trial was 120 sec at the end of which the rats were removed The retest latencies were calculated as the difference between the first and second latency time Better performance was indicated by longer retest latencies

Acetylcholine and Choline (Ch) Determination

ACh and Ch levels in the cerebral cortex, hippocampus and striatum were measured immediately after the retest of the passive avoidance reponse, 90 min after the treatment with scopolamine and 120 after the cognition-enhancing agent, respectively The rats were killed by head focussed microwave irradiation Microwave output was 1 3 kW and exposure time 5 sec The skull was opened, the brain removed and the frontoparietal cortex, hippocampus and in some cases the striatum dissected out and homogenized in a 0 4 N $HClO_4$ for 14 sec using an Ultraturrax apparatus The homogenate was centrifuged at 35,000 × g for 30 min at 0°C ACh and Ch content in the supernatant was measured by a gas chromatographic method [12]

Briefly, 10 nmol of the internal standard butyrrylcholine (BuCh) (Sigma) and 0 18 ml of ice-cold potassium acetate solution (7 5 N) were added to the supernatant Samples were then centrifuged at 35,000 × g for 30 min at 0°C To the supernatant 0 05 ml tetraethylammonium chloride (10 mM) (Eastman) plus 3 ml of 2% saturated ice-cold reineckate (Eastman) were added The samples were left on ice for 45 min and then centrifugated at 1,800 rpm for 20 min at 0°C The supernatant was discarded and the precipitate was freeze-dried To the dried residue, 0 6 ml of silver p-toluene sulphonate (5 mM) (Eastman) in acetonitrile (Aldrich) were added Samples were centrifugated for 10 min at 1,800 rpm at room temperature The supernatants were transferred to 1 ml-Kontes reaction vials and evaporated to dryness under a nitrogen stream 0 5 ml of 0 57 M propanyl chloride (Aldrich) in acetonitrile were added and the samples were heated for 20 min at 80°C After cooling, the samples were evaporated to dryness under a nitrogen stream 0 2 ml of 50 mM sodium benzenthiolate in acid methyl ethylketone (Fischer) was added to the samples which were then heated for 45 min at 80°C Subsequently, they were extracted in 0 02 ml of 0 5 M citric acid plus 0 2 ml of n-pentane (Fischer) The organic phase was discarded and n-pentane washing was repeated After removal of n-pentane traces with a nitrogen stream, amines were extracted in 0 1 ml of ethylacetate (Merck) after adding 0 02 ml of ammonium citrate 2 M ammonium hydroxide 7 5 M One μl of the samples was injected in an HP Sigma 3B gas chromatograph equipped with a nitrogen-phosphorus detector The previously silanized column was packed with 10% Pennwalt 233 (BDH) Gas chromatographic conditions were the following N_2 carrier gas flow 20 ml/min, oven, injector and detector temperature 145, 230 and 250°C, respectively Under these conditions the retention times for the demethylated analogs of ACh, Ch and BuCh were 100, 150 and 210 sec, respectively ACh and Ch standard curves were constructed from the peak height ratios of the demethy-

TABLE 3

EFFECT OF OCIRACETAM AND SCOPOLAMINE-INDUCED DECREASE IN BRAIN ACETYLCHOLINE LEVELS

Treatment	Dose mg/kg IP	N Rats	ACh levels (nm/g ± S E) Cortex	Hippocampus	Striatum
Saline	—	10	19 4 ± 0 2	24 3 ± 2 1	73 8 ± 8 6
Oxiracetam	50	6	18 4 ± 2 0	26 3 ± 3 1	75 4 ± 8 6
Scopolamine	0 63	11	7 0 ± 0 8*	10 7 ± 0 6*	42 4 ± 2 1*
Oxiracetam	10	6	7 3 ± 0 8	10 4 ± 0 5	44 9 ± 3 4
+ scopolamine	0 63				
Oxiracetam	50	6	12 3 ± 0 6†	18 9 ± 0 5†	44 8 ± 5 3
+ scopolamine	0 63				
Oxiracetam	100	5	19 0 ± 1 3†	18 2 ± 0 7†	42 3 ± 3 9
+ scopolamine	0 63				
Oxiracetam	300	8	8 6 ± 0 7	13 4 ± 1 9	43 2 ± 6 7
+ scopolamine	0 63				

*$p<0.01$ from saline, †$p<0.01$ from scopolamine alone

lated analogs to the peak height of the internal standard BuCh The resulting ratio was plotted against ACh and Ch concentrations Standard curves were linear between 1 and 30 nmol of ACh or Ch The quantity of ACh and Ch in the samples calculated from the standard curves were expressed as nmol per g of fresh tissue

Drugs

Oxiracetam was supplied by I S F (Trezzano sul Naviglio, Italy) Aniracetam was a gift by Hoffman-La Roche (Basel, Switzerland)

Oxiracetam was dissolved in saline and administered IP in a final volume of 0 5 ml Aniracetam was dissolved in the vehicle (0 3% v/v Tween-80 in saline) and administered PO in a final volume of 0 5 ml Scopolamine HBr (BDH) dissolved in saline was administered IP in a volume of 0 1 ml

Analysis of Data

Statistical significance of changes in ACh levels and passive avoidance responses was assessed by means of ANOVA with multiple comparisons according to BMDP Biomedical Computer Programs and the *t*-test

TABLE 4

EFFECT OF ANIRACETAM ON SCOPOLAMINE-INDUCED DECREASE IN BRAIN ACETYLCHOLINE LEVELS

Treatment	Dose mg/kg OS	N Rats	ACh levels (nm/g ± S E) Cortex	Hippocampus
Saline	—	10	19 4 ± 0 2	24 3 ± 2 1
Aniracetam	100	7	21 6 ± 1 9	23 8 ± 3 4
Scopolamine	0 63	11	7 0 ± 0 8*	10 7 ± 0 6*
Aniracetam	50	5	8 2 ± 1 2	11 4 ± 1 8
+ scopolamine	0 63			
Aniracetam	100	6	7 9 ± 0 3	16 1 ± 0 8†
+ scopolamine	0 63			
Aniracetam	300	5	7 6 ± 0 5	17 4 ± 1 3‡
+ scopolamine	0 63			

*$p<0.01$ from saline, †$p<0.05$ and ‡$p<0.01$ from scopolamine alone

RESULTS

The I latency time of saline treated rats was 13±4 sec (n=16) and was not modified by drug administration Table 1 shows that scopolamine (0 63 mg/kg IP) disrupted passive avoidance conditioned response acquisition as indicated by the short retest latency The dose of scopolamine was chosen on the basis of previous experience [6] Oxiracetam 50 mg/kg IP administered 30 min before scopolamine completely prevented the disruption in acquisition Oxiracetam at the dose of 100 mg/kg IP was less effective but still afforded a significant protection No effect was observed with the doses of 10 and 300 mg/kg IP Aniracetam administered orally only prevented the disruption of the passive avoidance at the dose of 100 mg/kg and was inactive at 50 and 300 mg/kg as shown in Table 2 Oxiracetam at the dose of 50 and 100 mg/kg IP and aniracetam at the dose of 100 mg/kg PO administered alone did not modify the retest latency as shown in Tables 1 and 2

The administration of scopolamine also brought about a 64, 56 and 42% decrease in cortical ACh level in the cerebral cortex, hippocampus and striatum respectively as shown in Table 3 Pretreatment with oxiracetam 50 and 100 mg/kg IP significantly reduced in the cortex and hippocampus the decrease in ACh level induced by scopolamine Oxiracetam exerted no effect on the striatum

The oral administration of aniracetam (100 and 300 mg/kg) significantly reduced scopolamine-induced decrease in ACh level in the hippocampus only as shown in Table 4

Oxiracetam at the dose of 50 and 100 mg/kg IP and aniracetam at the dose of 100 mg/kg PO administered alone did not affect ACh level as shown in Tables 3 and 4

None of the drugs tested modified choline levels in the brain regions investigated

DISCUSSION

Our experiments demonstrate that oxiracetam and aniracetam affect ACh levels in the cerebral cortex and hippocampus since they attenuate the decrease in ACh level induced by scopolamine According to our previous experience [6] the effect of scopolamine on brain ACh levels lasts 120 min with a maximum between 45 and 90 min For this reason the training test was carried out 60 min and the retest and ACh determination 90 min after scopolamine administration Scopolamine decreases brain ACh level by removing an inhibitory presynaptic muscarinic control on ACh release [9] The ensuing increase in ACh efflux is associated with a decrease in steady-state level even if ACh turnover is stimulated by anticholinergic drugs [13]

There is no evidence that aniracetam and oxiracetam displace scopolamine from pre and postsynaptic muscarinic receptors It may be therefore assumed that they reduce ACh decrease by stimulating its synthesis It has been shown that administration of large doses of choline also prevents atropine induced decrease in brain ACh level by facilitating ACh formation [24] The finding that oxiracetam prevents electroshock-induced decrease in cortical and hippocampal ACh level [23] also suggests an effect of this drug on ACh formation However a direct action on the basic steps of ACh synthesis is difficult to reconcile with the observation that oxiracetam did not modify the decrease in ACh level induced by scopolamine in the striatum in which the rate of ACh synthesis is much larger than in the neocortex [17] The possibility that oxiracetam might stimulate indirectly the activity of cholinergic neurons impinging on the cerebral cortex and hippocampus must be considered

The decrease in brain ACh and the amnesic effect of scopolamine are parallel effects caused by the blockade of the pre and postsynaptic muscarinic receptors respectively However, the doses of oxiracetam which antagonized the effect of scopolamine on cortical and hippocampal ACh levels also antagonized the disruption of the passive avoidance conditioned response A bell-shaped dose-effect relationship was obtained for both effects similar to that described for most behavioural responses induced by cognition-enhancing agents [22] A rough relationship seems therefore to exist between the stimulatory effect on cortical and hippocampal cholinergic mechanisms and the behavioural effect of oxiracetam Similarly, oxiracetam prevented both amnesia and the decrease in brain ACh induced by electroshock [23]

Aniracetam, which was given orally as a suspension, only attenuated scopolamine-induced ACh decrease in the hippocampus Whether this is due to differences in distribution or intrinsic pharmacological properties of the two drugs needs to be clarified Since most hippocampal ACh is contained in the nerve endings of the septo-hippocampal neurons [21], it appears that the functional integrity of these neurons is sufficient to permit the acquisition of a passive avoidance conditioned response However, at the dose of 300 mg/kg aniracetam did not prevent the disruption of the passive avoidance conditioned response but still reduced the decrease in hippocampal ACh level induced by scopolamine

Therefore our experiments have clearly demonstrated the effect of oxiracetam and aniracetam on central cholinergic mechanisms but other types of memory and other amnestic conditions must be investigated in order to firmly establish whether the cognition-enhancing properties of these drugs depend directly on brain cholinergic mechanisms

ACKNOWLEDGEMENT

This work was supported by CNR grant No CT 85 00325 04

REFERENCES

1 Banfi, S , L Dorigotti, M P Abbracchio and W Balduini Methylazoxymethanol microencephaly in rats neurochemical characterization and behavioral studies with the nootropic oxiracetam *Pharmacol Res Commun* **16** 67–83, 1984
2 Bartus, R T , R L Dean, III, K A Sherman, E Friedman and B Beer Profound effect of combining choline and piracetam on memory enhancement and cholinergic function in aged rats *Neurobiol Aging* **2** 105–111, 1981
3 Cumin, R , E F Bandle, E Gamzu and W E Haefely Effects of the novel compound aniracetam (Ro 13-5057) upon impaired learning and memory in rodents *Psychopharmacology (Berlin)* **78** 104–111, 1982
4 Davis, P A critical review of the role of the cholinergic system in human memory and cognition In *Memory Dysfunctions An Integration of Animal and Human Research From Preclinical and Clinical Perspectives*, edited by D S Olton *et al* New York New York Academy of Science, 1985, pp 212–217
5 Drachmann, D A and J Leavitt Human memory and the cholinergic system *Arch Neurol* **30** 113–129, 1974
6 Giarman, N J and G Pepeu The influence of centrally acting cholinolytic drugs on brain acetylcholine levels *Br J Pharmacol* **23** 123–130, 1964
7 Giurgea, C E Vers une pharmacologie de l'activite integrative du cerveau Tentative du concept nootrope en psychopharmacologie Actualites Pharmac, 25eme serie, Masson, Paris, pp 115–156, 1972
8 Giurgea, C E *Fundamentals to a Pharmacology of the Mind* Springfield, IL Charles C Thomas Publ , 1981, pp 124–186
9 Hadhazy, P and J C Szerb The effect of cholinergic drugs on (^{3}H) acetylcholine release from slices of rat hippocampus, striatum and cortex *Brain Res* **123** 311–322, 1977
10 Ketchum, J S , F R Sidell, E B Crowell, Jr , G K Aghajanian and A H Hayes, Jr Atropine, scopolamine and ditran comparative pharmacology and antagonists in man *Psychopharmacologia* **28** 121–145, 1973
11 Jarvik, M E and R Kopp An improved one-trial passive avoidance learning situation *Psychol Rev* **21** 221–224, 1967
12 Jenden, D J and I Hanin Gas chromatographic microestimation of choline and acetylcholine after N-demethylation by sodium benzenthiolate In *Choline and Acetylcholine Handbook of Chemical Assay Methods*, edited by I Hanin New York Raven Press, 1974, pp 135–150
13 Lundholm, B and B Sparf The effect of atropine on the turnover of acetylcholine in the mouse brain *Eur J Pharmacol* **32** 287–292, 1975
14 Pazzagli, A and G Pepeu Amnesic properties of scopolamine and brain acetylcholine in the rat *Int J Neuropharmacol* **4** 291–299, 1964
15 Pedata, F , F Moroni and G Pepeu Effect of nootropic agents on brain cholinergic mechanisms *Clin Neuropharmacol* **7** Suppl 1, New York Raven Press, 1984, pp 772–773
16 Pugsley, T A , Y -H Shih, L Coughenour and S F Stewart Some neurochemical properties of pramiracetam (CI-879), a new cognitive enhancing agent *Drug Dev Res* **3** 407–420, 1983
17 Racagni, G , D L Cheney, M Trabucchi, C Wang and E Costa Measurement of acetylcholine turnover rate in discrete areas of rat brain *Life Sci* **15** 1961–1975, 1976

18 Saletu, B and J Grunberger The hypoxia model in human psychopharmacology neurophysiological and psychometric studies with aniracetam i v *Hum Neurobiol* **3**· 171–181, 1984
19 Saletu, B , L Linzmayer, J Grunberger and H Pietshmann Double-blind, placebo-controlled, clinical, psychometric and neurophysiological investigations with oxiracetam in the organic brain syndrome of late life *Neuropsychobiology* **13** 44–51, 1985
20 Sansone, M , C Castellano and M Ammassari-Teule Improvement of avoidance acquisition by the nootropic drug oxiracetam in mice *Arch Int Pharmacodyn* **275**· 86–92, 1985
21 Sethy, V H , M J Kuhar, R H Roth, M H Van Woert and G K Aghajanian Cholinergic neurons effect of acute septal lesion on acetylcholine and choline content of rat hippocampus *Brain Res* **55** 481–484, 1973
22 Schindler, U , D K Rush and S Fielding Nootropic drugs animal models for studying effects on cognition *Drug Dev Res* **4** 567–576, 1984
23 Spignoli, G and G Pepeu Oxiracetam prevents electroshock-induced decrease in brain acetylcholine and amnesia *Eur J Pharmacol* **126** 253–257, 1986
24 Wecker, L , W D Dettbarn and D E Schmidt Choline administration modification of the central actions of atropine *Science* **199**· 86–87, 1978
25 Wurtman, R J , S G Magil and D K Reinstein Piracetam diminishes hippocampal acetylcholine levels in rats *Life Sci* **28** 1091–1093, 1981