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Reversal of Amnesia by an Orally Active ACTH 4-9 Analog (Org 2766)

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RIGTER, H., R. JANSSENS-ELBERTSE AND H. VAN RIEZEN. *Reversal of amnesia by an orally active ACTH 4-9 analog (Org 2766).* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 53–58, 1976. – The ACTH 4-9 analog, H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH (Org 2766), attenuates in rats CO_2-induced amnesia for a one-trial passive avoidance step-through response when administered prior to the retrieval test but not when given prior to acquisition. Even a dose of 0.001 μg/rat Org 2766 yields an anti-amnesic effect. In this respect Org 2766 is more active than the ACTH fragment ACTH 4-10. An anti-amnesic effect was also obtained when Org 2766 was administered orally. ACTH 4-10 (100 μg/rat) has to be given SC within 8 hr of the retrieval test in order to be effective. A similar time span of effectiveness was observed when Org 2766 was SC injected in a dose of 0.1 μg/rat. The anti-amnesic effect of ACTH 4-10 remains when the time interval between acquisition and retrieval is extended beyond the usual 24 hr. The same appeared to be true for SC administered Org 2766. It is suggested that ACTH-like peptides, and particularly the orally active Org 2766, may be helpful in the treatment of deficient mental performance.

ACTH Retrograde amnesia CO_2 Memory retrieval

NUMEROUS studies have shown that pituitary hormones influence behavior in the rat independently from their effect on endocrine target organs. The behavioral effect of adrenocorticotrophic hormone (ACTH), for example, is not dependent on an ACTH-induced release of corticosteroids from the adrenal cortex since a number of ACTH analogs which are virtually devoid of adrenocorticotrophic activity, are equally able to influence behavior. Thus, melanocyte-stimulating hormone (MSH), which shares thirteen amino acids with ACTH, exerts behavioral effects similar to those of ACTH [9,12]. Even the fragments ACTH 4-10, ACTH 4-9, ACTH 4-8 and ACTH 4-7 show the behavioral activity of the parent hormone [9,28]. Structural modifications of these amino acid sequences may lead to changes in the behavioral effects. Thus, the behavioral potency of the ACTH 4-9 analog, H-Met(O)-Glu-His-Phe-D-Lys-Phe-OH, is increased a thousand-fold compared with ACTH 4-9 [9,28]. In this peptide 4-methionine has been substituted by 4-methionine sulfoxide [Met(O)], 8-arginine by 8-D-lysine and 9-tryptophan by 9-phenylalanine. The increase in behavioral potency due to this combination of substitutions correlates with an increase in *in vitro* half-life of the peptide which may be ascribed to an increased resistance against biotransformation [30].

The behavioral activity of ACTH analogs has been assessed in a variety of tests. Hypophysectomy in rats interferes with acquisition of shock-motivated active and passive avoidance responses in rats. Behavior can be restored by administration of ACTH or ACTH-analogs [9,14]. We have found that acquisition of a watermaze habit is similarly disrupted by hypophysectomy. The deficient acquisition can be normalized by ACTH 4-10 [17].

Treatment with ACTH or ACTH-analogs as a rule does not alter the rate of acquisition of shock-motivated active avoidance responses in intact rats [13,16]. We have observed a similar lack of effect on the acquisition of a watermaze habit by intact rats (Rigter, unpublished results). It is possible that during acquisition of avoidance behavior sufficient endogenous ACTH is mobilized to enable the animals to optimally cope with the task. Under some conditions, however, ACTH or MSH may improve acquisition [2,25]. The influence of ACTH and MSH on acquisition of appetite-motivated responses has been studied less extensively. Guth *et al.* [10] showed that administration of ACTH during acquisition of an appetite-motivated response increased lever press responding late in the training period. Kastin *et al.* [12] reported that MSH facilitated the acquisition of food-rewarded behavior in a multiple T-maze.

Administration of ACTH, ACTH analogs or MSH to rats during the period of extinction of behavioral responses results in a delay of extinction. This is true for shock-motivated avoidance responses [9,27], for food-motivated responses [8,12], and for sexual-motivated behavior [3]. We have studied the effects of ACTH, ACTH analogs and MSH on the extinction of avoidance behavior not motivated by electric shock. With the so-called conditioned taste aversion (or bait shyness) paradigm rats were subjected to the unpleasant effects of an intraperitoneal injection of lithium chloride after drinking a novel sugar water solution. This resulted in a long-lasting avoidance of sugar water. ACTH, ACTH 4-10 and MSH appeared to delay extinction of this avoidance behavior [19,21].

In another series of experiments it has been demonstrated that ACTH, ACTH 4-10 and MSH attenuate carbon dioxide-induced amnesia for a passive avoidance response when administered prior to the retrieval test but not when

given prior to acquisition and the induction of amnesia [18,23]. This effect is independent of the nature of the amnesic agent and the behavioral test as a similar facilitation of retrieval could be obtained in a study on electroconvulsive shock-induced amnesia for an appetite-motivated response [22]. Rigter *et al.* [20] found that carbon dioxide-induced amnesia for a passive avoidance response remains present over a 2 week period. Irrespective of the duration of the acquisition-test interval, pre-retrieval administration of $ACTH_{4-10}$ results in an attenuation of amnesia. Consistent with the view that ACTH and related peptides facilitate retrieval is the finding that ACTH, ACTH analogs and MSH potentiate the retrieval of weak passive avoidance responses [6,9].

De Wied and associates have reported that the ACTH 4-9 analog, H-Met-(O)-Glu-His-Phe-D-Lys-Phe-OH, delays the extinction of a pole jump avoidance response. The behavioral potency of this peptide is increased a thousand-fold compared to ACTH 4-10 [9, 28, 30].

The effects of a similar peptide have been studied in a number of behavioral tests. Since the sulfoxide in H-Met(O)-Glu-His-Phe-D-Lys-Phe-OH contains a centre of assymetry and therefore gives rise to a mixture of 2 stereoisomers, we preferred to use the corresponding sulfon. This peptide, i.e., H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH (Org 2766), is virtually as active with respect to its effect on the extinction of the pole jump avoidance response as the sulfoxide (Greven and De Wied, personal communication). Elsewhere, it was reported that SC administered Org 2766 like ACTH 4-10 restores the deficient acquisition of a watermaze habit in hypophysectomized rats [17] and delays the extinction of a conditioned taste aversion [19]. In the present paper we will focus on the effect of Org 2766 on carbon dioxide (CO_2)-induced amnesia. In a first experiment the anti-amnesic effect of subcutaneously administered Org 2766 was studied.

METHOD

One hundred and ten male Wistar rats weighing 230–240 g were used. They were obtained from the TNO breeding station, Zeist, The Netherlands, 14 days before the start of the experiment. The rats were trained in a step-through passive avoidance apparatus of the type described by Ader *et al.* [1]. In short, this consisted of a chamber with black walls and a grid floor. A brightly lit, elevated runway protruded from the front wall. A scrambled foot shock could be delivered through the grid floor of the chamber. Eleven groups of 10 rats were used. They were given 3 pretraining trials on Day 1. A pretraining trial consisted of placing a rat at the end of the runway facing an opening in the front wall of the chamber. The time taken for the animal to enter the chamber (step-through latency) was recorded. On Day 2, a single acquisition trial was run. This was identical to the pretraining trials with the exception that 0.5 mA footshock (FS) was given for 3 seconds after the rat had entered the chamber. Eight groups were treated with 100% CO_2 immediately after footshock (FS-CO_2 groups). Three other groups did not receive CO_2 (FS-NoCO_2 groups). A single test trial was given 24 hr after acquisition; the maximal duration of this retrieval trial was 300.0 sec. The results were analyzed by means of the Yates test [31]. The test scores were divided in 3 classes: (1) latencies of 0 10.0 sec (no avoidance); (2) latencies of 10.1 - 299.9 sec (incomplete avoidance); (3) latencies of 300.0 sec (refusal to enter the chamber; complete avoidance).

Org 2766 was dissolved in distilled water containing 5% Mannitol. The vehicle was used as placebo. Three doses of Org 2766 were administered: 0.1 μg, 1 μg or 10 μg/rat SC, either 1 hr prior to acquisition or 1 hr prior to retrieval. The design of the experiment and the results are given in Table 1.

TABLE 1

REVERSAL OF CO_2-INDUCED AMNESIA FOR A ONE-TRIAL PASSIVE AVOIDANCE RESPONSE BY SC ADMINISTERED ORG 2766

Group	Treatment 1 hr prior to		Percentage of rats showing		
	acquisition	retrieval	no avoidance	incomplete avoidance	complete avoidance
FS-CO_2	placebo	placebo	60	40	0
FS-CO_2	0.1 μg Org 2766	placebo	40	60	0
FS-CO_2	1.0 μg Org 2766	placebo	60	30	10
FS-CO_2	10.0 μg Org 2766	placebo	40	40	20
FS-CO_2†	placebo	0.1 μg Org 2766	10	60	30
FS-CO_2*	placebo	1.0 μg Org 2766	20	60	20
FS-CO_2†	placebo	10.0 μg Org 2766	20	40	40
FS-CO_2*	10.0 μg Org 2766	10.0 μg Org 2766	20	50	30
FS-NoCO_2‡	placebo	placebo	0	10	90
FS-NoCO_2‡	10.0 μg Org 2766	placebo	0	0	100
FS-NoCO_2‡	placebo	10.0 μg Org 2766	0	10	90

FS: foot shock; CO_2: amnesic treatment with CO_2; NoCO_2: sham amnesic treatment.
Significant differences to the upper group (FS-CO_2, placebo-placebo), using one-tailed Yates test.
*$p<0.05$.
†$p<0.01$.
‡$p<0.001$.

RESULTS AND DISCUSSION

In the placebo-treated FS-CO_2 group amnesia is reflected by the absence of substantial avoidance. Org 2766 did not affect amnesia when injected prior to the acquisition trial. However, all doses of Org 2766 attenuated amnesia when the peptide was given 1 hr prior to retrieval. This pattern of activity is identical to that of ACTH 4-10 [23]. The present study did not attempt to determine the effectiveness of Org 2766 relative to ACTH 4-10. However, the results suggest that SC doses of lower than 0.1 μg/rat still may be effective whereas the threshold of an anti-amnesic effect of ACTH 4-10 is between 0.1 and 1 μg/rat (Rigter, unpublished results).

In a second experiment we injected Org 2766 SC in dosages of 0.1, 0.01 and 0.001 μg/rat 1 hr prior to the retrieval test. As can be seen from Table 2, even a dosage of 0.001 μg Org 2766 attenuated CO_2-induced amnesia for the one-trial passive avoidance response, although the effect was borderline.

We subsequently studied the possibility that Org 2766 may be orally active in the amnesia test. One hundred and ten male Wistar rats, weighing 200–230 g, were used. Ten groups of 10 rats were subjected to footshock followed by CO_2-treatment (FS-CO_2 groups). Of these groups, one group was given a placebo tablet (containing 1% magnesium stearate; 10% potato starch and 89% mannitol) 30 min prior to retrieval whereas the other groups received tablets containing 10, 100 or 1000 μg Org 2766 either 30, 60 or 120 min prior to retrieval. One group of 10 rats was subjected to footshock but not to CO_2 (FS-NoCO_2) and was treated with a placebo tablet 30 min before the test. The results are given in Table 3.

The oral administration of a placebo tablet did not affect amnesia or passive avoidance. When Org 2766 was given 30 min prior to the test, no effect on CO_2-induced amnesia could be detected. Only the highest dose of Org 2766 exerted an anti-amnesic effect when given 60 min before retrieval. A treatment-test interval of 2 hr was most effective: all doses reduced amnesia, 1000 μg being more

TABLE 2

ANTI-AMNESIC EFFECT OF SC INJECTED ORG 2766

Group	Treatment 1 hr prior to retrieval	Percentage of rats showing no avoidance	incomplete avoidance	complete avoidance
FS-CO_2	placebo	90	10	0
FS-CO_2†	0.1 μg Org 2766	20	40	40
FS-CO_2*	0.01 μg Org 2766	30	30	40
FS-CO_2‡	0.001 μg Org 2766	60	30	10
FS-NoCO_2†	placebo	0	26.6	73.2

*$p<0.01$.
†$p<0.001$.
‡$p<0.053$ (compared with the placebo-treated FS-CO_2 group). FS: foot shock; CO_2: amnesic treatment; NoCO_2: sham treatment. Ten rats per group, with the exception of FS-NoCO_2 (n = 8).

TABLE 3

REVERSAL OF CO_2-INDUCED AMNESIA FOR A ONE-TRIAL PASSIVE AVOIDANCE RESPONSE BY ORALLY ADMINISTERED ORG 2766

Group	Treatment	Time of treatment prior to retrieval (min)	Percentage of rats showing no avoidance	incomplete avoidance	complete avoidance
FS-CO_2	placebo	30	80	20	0
FS-CO_2	10 μg Org 2766	30	50	50	0
FS-CO_2	10 μg Org 2766	60	70	20	10
FS-CO_2*	10 μg Org 2766	120	40	60	0
FS-CO_2	100 μg Org 2766	30	80	10	10
FS-CO_2	100 μg Org 2766	60	50	50	0
FS-CO_2 †	100 μg Org 2766	120	30	50	20
FS-CO_2	1000 μg Org 2766	30	50	50	0
FS-CO_2*	1000 μg Org 2766	60	50	30	20
FS-CO_2‡	1000 μg Org 2766	120	20	40	40
FS-No-CO_2‡	placebo	30	0	30	70

*$p<0.05$.
†$p<0.01$.
‡$p<0.001$ (compared with the placebo-treated FS-CO_2 group).

TABLE 4

TIME SPAN OF EFFECTIVENESS OF S.C. ADMINISTERED ORG 2766

Group	Treatment	Time of treatment prior to retrieval (hr)	Percentage of rats showing no avoidance	incomplete avoidance	complete avoidance
FS-CO_2	placebo	1	80	20	0
FS-CO_2†	0.1 μg Org 2766	1	20	40	40
FS-CO_2†	0.1 μg Org 2766	2	20	30	50
FS-CO_2†	0.1 μg Org 2766	4	30	30	40
FS-CO_2†	0.1 μg Org 2766	6	30	50	20
FS-CO_2	0.1 μg Org 2766	8	60	40	0
FS-CO_2	0.001 μg Org 2766	1	60	30	10
FS-CO_2*	0.001 μg Org 2766	2	50	30	20
FS-CO_2	0.001 μg Org 2766	4	70	20	10
FS-CO_2	0.001 μg Org 2766	6	80	10	10
FS-CO_2	0.001 μg Org 2766	8	70	30	0
FS-$NoCo_2$‡	placebo	1	0	40	60

*$p<0.05$.
†$p<0.01$.
‡$p<0.001$ (compared with the placebo- treated FS-CO_2 group).

effective than 10 μg. It can be concluded that oral administration of Org 2766 attenuates amnesia. In comparison to SC administration, higher doses and longer treatment-test intervals are required.

We have previously reported that 100 μg/rat ACTH 4-10 had an anti-amnesic effect when the peptide was injected SC within 8 hr prior to the retrieval test. One might assume that the behaviorally more active peptide Org 2766 would yield a longer time span of effectiveness. This hypothesis was not confirmed in an experiment in which either 0.1 or 0.001 μg/rat Org 2766 was injected SC 1, 2, 4, 6 or 8 hr prior to the retrieval test in the passive avoidance paradigm. The design of the experiment and the results are presented in Table 4.

It appeared that 0.1 μg Org 2766 had to be injected SC within 8 hr of retrieval in order to yield an anti-amnesic effect. The effective time span was reduced to less than 4 hr when a SC dosage of 0.001 μg/rat was used. These data do not yet justify a conclusion about a (dis)similarity between the time-response relationships of ACTH 4-10 and Org 2766. More insight in the relation between dose and duration of effect is required.

CO_2-induced amnesia for the passive avoidance response remains present over a period of at least 2 weeks. Irrespective of the duration of the acquisition-test interval, preretrieval administration of ACTH 4-10 results in an attenuation of amnesia [20]. For Org 2766 a similar finding has been obtained. Four groups of 10 male Wistar rats were subjected to footshock and amnesic treatment and tested for retrieval 14 days later. Another group of 10 rats was subjected to footshock but not to CO_2. The animals were injected SC with either 0.001, 0.1 or 10 μg/rat Org 2766 or placebo 1 hr before the retrieval test according to the schedule given in Table 5.

Table 5 shows that amnesia was present in the placebo-treated FS-CO_2 group 14 days after the acquisition trial and the anmesic treatment. The anti-amnesic effect of Org 2766 also remained present: the peptide attenuated amnesia in a dose-dependent manner, although somewhat higher doses may be needed than when the acquisition-test interval is set at 24 hr (cf. Tables 1 and 2). Our findings clearly indicate that Org 2766 is able to attenuate amnesia in rats and resembles in this respect other ACTH fragments and analogs, such as ACTH 4-10. The oral effectiveness of Org 2766 opens perspectives for its use in human pharmacology.

Recently, SC administered ACTH 4-10 has been shown to modulate behavioral and EEG responses in human volunteers. Miller *et al.* [15] found that ACTH 4-10 delays the development of EEG signs of habituation in a disjunctive reaction time task. Subjects were presented with two different warning signals. One signal indicated that the subjects had to respond to a subsequent stimulus by pressing a bar (response trials); the other signal indicated that the subjects had to abstain from pressing (nonresponse trials). The ACTH 4-10-treated group did not show habituation to the response signal but habituated normally to the nonresponse signal, thus giving evidence of an increase in selective attention. These authors also reported that ACTH 4-10-treated volunteers performed better on a Benton Visual Retention Test. This latter finding was confirmed by Sandman *et al.* [24], who also noticed that their peptide-treated subjects were more attentive to the stimuli in a visual discrimination experiment. They suggested that ACTH 4-10 improves processes mediating selective attention. Such a view is consistent with the results of Gaillard and Sanders [7]. They found that ACTH 4-10 improves performance of subjects in a continuous reaction time task demanding a sustained level of attention.

In our opinion, the view that ACTH-like peptides improve (selective) attention may also explain the improvement of retrieval by ACTH 4-10 and Org 2766 in the amnesia paradigm.

Behavior consists of a chain of learned or genetically determined motor patterns (programs). The individual links (programs) are selected on basis of an evaluation of perceptive input and memory of previous experiences and coupled to each other to produce the smoothly running

TABLE 5

ANTI-AMNESIC EFFECT OF S.C. ADMINISTERED ORG 2766 AFTER AN ACQUISITION-TEST INTERVAL OF 14 DAYS

Group	Treatment	Percentage of rats showing no avoidance	incomplete avoidance	complete avoidance
FS-CO_2	placebo	90	10	0
FS-CO_2	0.001 μg Org 2766	90	10	0
FS-CO_2*	0.1 μg Org 2766	50	50	0
FS-CO_2†	10.0 μg Org 2766	30	40	30
FS-NoCO_2‡	placebo	20	20	60

*$p<0.05$.
†$p<0.01$.
‡$p<0.001$ (compared with placebo-treated FS-CO_2 group). Acquisition-test interval was 14 days. Treatment was given 1 hr before test.

process of controlled behavior. The control depends mainly on two brain functions. One is the capacity of the brain to retrieve and activate these programs. The second is the process of selecting the correct program. Human experiments on mental control capacity suggest that both the selection capacity and the retrievability of programs are under motivational control and that this control mechanism is located in the non-specific reticulo-thalamic system [11]. We propose that ACTH (-fragments) activate this mechamism. Lesion studies [4], implantation studies [29] and recent electrophysiological work [26] suggest that one locus of action of ACTH-like peptides is in the non-specific reticulo-thalamic system.

The two functions of the retrieval and selection capacity decline in aging subjects. It might be argued that the cause of this decline is a deficiency of the humoral control of these functions. The findings that mental deficits in hypopituitary patients are similar to those in the aged [5], supports this view. If so, ACTH-analogs (in particular the orally active Org 2766) may be useful in the treatment of mental problems associated with senescence. They may also prove to be useful in other conditions where retrieval and/or selection capacity are deficient.

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