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Chlordiazepoxide-Induced Potentiation of Hexobarbitone Sleeping Time is Reduced by $ACTH_{(1-24)}$

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VELLUCCI, S. V. *Chlordiazepoxide-induced potentiation of hexobarbitone sleeping time is reduced by* $ACTH_{(1-24)}$. PHARMACOL BIOCHEM BEHAV **21**(1) 39–41, 1984.—The effects of $ACTH_{(1-24)}$ on the potentiation of hexobarbitone sleeping time by chlordiazepoxide (CDP) were investigated in the rat. CDP (10 mg/kg, IP) significantly ($p<0.001$) potentiated hexobarbitone (100 mg/kg, IP)-induced sleeping time. This effect was significantly ($p<0.01$) reduced by $ACTH_{(1-24)}$ (10 μg/100 g, IP), although ACTH did not influence the response to hexobarbitone in the absence of CDP. The possible implications of these findings are discussed in relation to recent observations concerning the possible interactions between ACTH and benzodiazepines.

ACTH Benzodiazepines Hexobarbitone sleeping time Rat

IT is well established that ACTH can exert a variety of effects on the central nervous system (e.g., effects on behaviour) that are independent of its peripheral steroidogenic activity [8, 9, 23]. The existence of ACTH and related peptides which are not of pituitary origin has been demonstrated in the CNS [1, 24, 25, 26] and tends to support the contention that these peptides have a physiological function in the brain.

ACTH and related peptides administered centrally or peripherally have an anxiogenic effect [12, 13, 14] and are capable of antagonizing the anxiolytic effects of chlordiazepoxide in two animal models of anxiety, namely the active social interaction test [14] and the Geller-Seifter rat conflict test [33]. ACTH peptides have also been shown to antagonize the electrophysiological effects of benzodiazepines [22] and to reverse diazepam's antileptazol effect in mice [32]. Conversely benzodiazepines have been shown to block ACTH-induced excessive grooming [7].

Thus ACTH peptides have been shown to antagonize the anxiolytic, sedative and anticonvulsant properties of benzodiazepines, and although the precise nature of this interaction has not yet been established, it was of interest to determine whether ACTH also had an effect on the benzodiazepine-induced potentiation of hexobarbitone sleeping time in the rat.

METHOD

Male Lister hooded rats (home-bred, 200–250 g) were housed 2/cage in a room maintained at 22°C, with an 11–13 hr light-dark cycle (lights on at 07.00). Food and water were freely available. To minimise the stress of injection which would evoke ACTH and glucocorticoid release, the rats were handled once daily for 10 days prior to use [19]. The experiments were carried out between 09.00 and 12.00, in the room where the animals were housed and each animal was used once only. For the determination of sleeping time, animals were pre-treated with drug or vehicle and then given hexobarbitone in a dose of 100 mg/kg, IP at the times stated. The duration of sleeping time was assessed using a standard procedure and was defined as the time from loss of the righting reflex until the return of the reflex (i.e., the righting reflex was tested at 10–15 sec intervals following hexobarbitone injection; once the reflex was absent, the animals were placed in the supine position until they were able to right themselves spontaneously).

The following drugs were used: Hexobarbitone sodium (May and Baker) was dissolved in 0.9% saline and administered in a dose of 100 mg/kg. Chlordiazepoxide hydrochloride (Roche Products Ltd) was dissolved in distilled water and administered in a dose of 10 mg/kg, 30 min prior to hexobarbitone. $ACTH_{(1-24)}$ (Synacthen, Ciba Laboratories) was dissolved in 0.9% saline in plastic vials, immediately prior to injection and administered in a dose of 10 μg/100 g, 5 min prior to hexobarbitone. Corticosterone (Sigma Chemical Co) was suspended in 5% ethanol solution in distilled water (v/v) and administered in doses of up to 12 mg/kg, 20 min before hexobarbitone.

All drugs were administered IP at the times stated, in a volume of 1 ml/kg, except corticosterone which was administered as 3 ml/kg. The controls received equal-volume injections of the appropriate vehicle.

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RESULTS

The mean hexobarbitone sleeping time was unaffected by vehicle injections. Thus the results obtained with hexobarbitone in the absence and presence of the different vehicle treatments have been grouped together. Hexobarbitone (100 mg/kg, IP) rapidly induced ataxia, and complete loss of the righting reflex was noted within 2–3 min of injection. Chlordiazepoxide (CDP, 10 mg/kg) administered 30 min prior to hexobarbitone, significantly ($p<0.001$) prolonged hexobarbitone sleeping time (Table 1). This potentiation was partially reversed ($p<0.01$) by the administration of $ACTH_{(1-24)}$ (10 μg/100 g, IP) 5 min prior to hexobarbitone. $ACTH_{(1-24)}$ given alone had no effect on hexobarbitone sleeping-time. The potentiation of hexobarbitone sleeping-time by CDP was unaffected if corticosterone (in doses of up to 12 mg/kg IP) or its vehicle were administered 20 min prior to hexobarbitone. CDP alone in the dose used here produced a moderate degree of ataxia, but did not cause loss of the righting reflex.

TABLE 1

THE EFFECT OF $ACTH_{(1-24)}$ ON CDP-INDUCED POTENTIATION OF HEXOBARBITONE SLEEPING TIME IN THE RAT

Group	Treatment	Sleeping Time (Min)	n
I	Hexobarbitone (100 mg/kg) Vehicles (1 ml/kg)	19.8 ± 0.85	24
II	CDP (10 mg/kg) Hexobarbitone (100 mg/kg)	49 ± 2.94**	14
III	CDP (10 mg/kg) $ACTH_{(1-24)}$ (10 μg/100 g) Hexobarbitone (100 mg/kg)	34.3 ± 3.8*†	10
IV	CDP (10 mg/kg) ACTH Vehicle (1 ml/kg) Hexobarbitone (100 mg/kg)	46 ± 3.1	6
V	$ACTH_{(1-24)}$ (10 μg/100 g) Hexobarbitone (100 mg/kg)	22.7 ± 2.69	7
VI	CDP (10 mg/kg) Corticosterone (12 mg/kg) Hexobarbitone (100 mg/kg)	45.8 ± 4.05	6
VII	CDP (10 mg/kg) Corticosterone Vehicle (3 ml/kg) Hexobarbitone (100 mg/kg)	43.8 ± 4.38	6
VIII	CDP (10 mg/kg)	(ataxia only)	6

CDP, Corticosterone and $ACTH_{(1-24)}$ were administered IP 30, 20, and 5 min before hexobarbitone, respectively.
Significantly different from I: **$p<0.001$; *$p<0.005$.
Significantly different from II: †$p<0.01$; (2-tailed t-test).

DISCUSSION

These results demonstrate that $ACTH_{(1-24)}$ is capable of significantly reducing the CDP-induced potentiation of hexobarbitone sleeping-time in the rat. Although the ACTH was administered peripherally, it is well-established that this peptide is still capable of exerting central effects in the dose used [14,23]. As $ACTH_{(1-24)}$ which has endocrine as well as behavioural activity was used, it is possible that the observed effect may have been due not to the ACTH itself but to concomitantly released adrenal glucocorticoids. However, this is considered unlikely, as the administration of corticosterone, the principal glucocorticoid in the rat, in doses which produce plasma corticosterone concentrations similar to those obtained following ACTH injection or severe stress, did not significantly influence the sleeping-time of the rats treated with CDP and hexobarbitone. Although there is no evidence to indicate that the drugs used in the present investigation interact pharmacokinetically in the short-term, one cannot completely exclude the possibility that the observed effects may be due to altered rates of uptake or metabolism of the drugs, rather than a direct interaction at the level of the CNS.

These results are in agreement with other published observations which indicate that ACTH and benzodiazepines have opposite behavioural, biochemical and electrophysiological effects [7, 14, 22, 32, 33]. These findings thus lend some support to the idea of the possible occurrence of a functional antagonism between ACTH and the benzodiazepines in the CNS. However, benzodiazepines do not antagonize all the behavioural effects of ACTH and vice versa. For example, one group of authors [11] have shown that ACTH-induced excessive grooming was unaffected by non-sedative doses of benzodiazepines, although such doses decreased the excessive grooming evoked by exposure to a novel environment. Neither did ACTH appear to have any effect on the drink latency test, a model of anxiety [13], although it has been suggested that the action of benzodiazepines on drinking does not necessarily correlate with their anti-anxiety effects [28].

In view of these differences between some of the behavioural effects of ACTH and benzodiazepines it is not surprising that ACTH and related peptides such as $ACTH_{(4-10)}$ do not appear to directly influence the binding of benzodiazepines to their receptors [6]. The benzodiazepine "antagonist" effects of ACTH may therefore be due to a non-specific action such as a generalized increase in arousal, motivation or fear [9, 15, 20], or possibly to a direct effect on opiate receptors. Certainly the opiate antagonist, naloxone, and ACTH peptides appear to have several behavioural and pharmacological properties in common. Thus ACTH peptides can antagonize morphine-induced analgesia [16,27], block the depressant effects of morphine on evoked potentials in the isolated spinal cord [34], have appreciable affinity for central opiate receptors [10, 29, 30, 31], cause hyperalgesia in high doses [2] and precipitate withdrawal symptoms in morphine-dependent rats [3,20]. Morphine itself has been shown to exert a dual action (i.e., to have both inhibitory and excitatory effects [21], with high doses of ACTH mimicking the latter. This excitatory effect of morphine was thought to be mediated by a non-stereospecific, non-naloxone reversible opiate receptor for which ACTH was suggested to be the endogenous ligand. This may offer a possible explanation as to why ACTH can reverse the effects of benzodiazepines. On the other hand, naloxone has been shown to block the anti-conflict activity of CDP [4] and to block ACTH- or novelty-induced excessive grooming [11, 17, 18]. Thus a more specific effect on opiate receptors may be involved.

The observation that ACTH in the absence of CDP did not affect hexobarbitone sleeping time is in agreement with that of other authors [5] who showed that $ACTH_{(4-10)}$ and

$ACTH_{(7-10)}$ were inactive in antagonizing pentobarbital-induced sleep, whether administered peripherally or centrally. The fact that ACTH reduced the potentiation by CDP of hexobarbitone sleeping-time but did not affect the response to hexobarbitone alone, may be indicative of a functional interaction between CDP and ACTH. On the other hand the proposed excitatory effects of ACTH may not have been potent enough to overcome the effects of hexobarbitone, whereas they could overcome the much weaker effects of CDP.

Thus the observed reduction, by ACTH, of the CDP-induced potentiation of hexobarbitone sleeping time in the rat may be due to a generalized excitatory effect of ACTH, which may be mediated via an action on opiate receptors (or a sub-population of opiate receptors), rather than to a specific interaction between benzodiazepines and ACTH at the level of the benzodiazepine receptor. Further studies which may help to resolve this question including a structure-activity study of ACTH peptides will be carried out.

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