which appear to interact with an acid grouping with a pKa of around 5. Also, for both types of blocker the effect of pH modifies this interaction in a predictable way when the pK's of the drugs and receptors are taken into account. It is not known if the amiloride binding site which controls sodium access in epithelia is a selectivity filter controlling entry to a carrier mechanism or whether it forms an integral part of a carrier.

The blocking effects of tetrodotoxin (and saxitoxin) have been demonstrated in a wide variety of excitable tissues in both vertebrates and invertebrates. Similarly, amiloride sensitive sodium channels are found not only in mammals, but in other vertebrates and lower forms such as insects and annelids[17]. The wide distribution of both types of channel and the similarities outlined in this report suggests they may have a common ancestry.

[17] A. W. CUTHBERT, in *Drugs and Transport Processes* (Ed. B. A. CALLINGHAM; Macmillan, London 1974), p. 173.

Conditioned Suppression: Dissociation of Learning in Baclofen Treated Rats

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Summary. In rats, baclofen induced a memory deficit related to a dissociation of learning. Baclofen given prior to training or prior to testing had no effect on the amnesia elicited by diazepam.

Some biochemical, electrophysiological or pharmacological effects induced by the benzodiazepines seem to be related to their actions on central GABA-(γ-aminobutyric acid)-ergic mechanisms[1]. The involvement of a GABA link in some behavioral effects of benzodiazepines was examined in a previous work[2], and it was found that baclofen – a compound structurally related to GABA – enhanced the food intake of rats placed in a non-familiar situation, as benzodiazepines did. The purpose of this work was to give some information about the role of a GABA-ergic mechanism in the amnesic effect of benzodiazepines[3]. With that aim, an amnesic effect, and a modification of diazepam-elicited amnesia eventually induced by baclofen, were investigated in rats.

Material and methods. The experiments were carried out on male Wistar A.F. rats (180–200 g). The animals were housed 8 per cage with free access to food and water, unless otherwise noted, and maintained in 12/12 h light-dark cycle. The test situation was a (36 × 36 × 30 cm) translucent box. A drinking bottle was located in a corner of the box. A metal drinking-tube terminated the bottle at a height of 3 cm above an electrifiable part of the floor (22 × 10 cm). The rats were deprived of water (but not of food) during the 16 h preceding their introduction into the test apparatus.

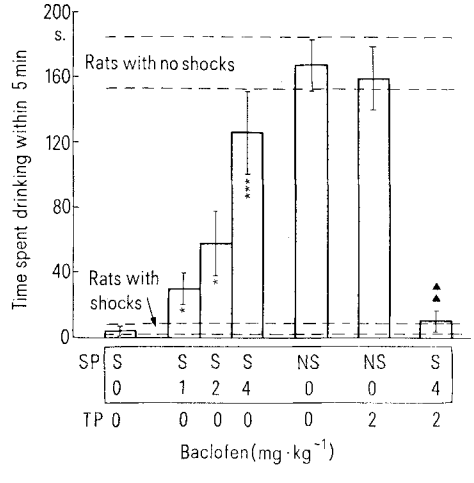

Fig. 1. 'Test phase'. Effects of baclofen given before either the 'shocks phase' (SP) or the 'test phase' (TP) on the drinking inhibition of rats. S, rats with shocks; NS, Rats with no shocks. *, **, ***, indicate that S treated rats differ as compared to S control rats at 0.05, 0.02 and 0.01 level respectively. ▲▲, indicate that S rats, given baclofen before both SP and TP differ at 0.02 level from animals given baclofen only before SP. Vertical bars represent SEM.

'Shocks phase'. Each rat was placed in the test situation and shocked (2 mA) as soon as it started to drink. After this electric shock, the animals remained 1 min in the apparatus, and each time they drank, they were shocked again. Previous experiments have shown that rats generally start drinking within 2 min and did not tolerate more than 2 shocks. The rats which do not drink within 2 min and which receive more than 2 shocks, were eliminated of the study. Rats with no shocks were given a 3 min placement in the apparatus without any shock.

'Test phase'. 4 days after the 'shocks phase', each rat was placed for 5 min in the test situation without any shock. During this period the time spent drinking was recorded to the nearest second with a manually operated chronometer.

Drugs were administered (1 ml/100 g) 30 min before the 'shocks phase' and/or the 'test phase'. Baclofen (β-parachlorophenyl-γ-aminobutyric acid) s.c. and diazepam i.p. were injected, as suspension with acacia gum. The statistical comparison between groups (10 to 12 rats per group) was done using the Student's t-test or Darmois' t-test.

Results. Control rats with shocks exhibit, as compared to control rats with no shocks, a marked drinking behavior inhibition (Figure 1). In rats treated with baclofen 30 min before the 'shocks phase', this drinking inhibition was reduced or even suppressed. Baclofen (2 mg.kg^{-1}) given 30 min before the 'test phase' abolished the effect of the administration of this drug before the 'shocks phase'. At this dose, baclofen had no effect on the drinking time either of rats with no shocks, or of rats with shocks (15 ± 7 sec); Figure 2 shows that baclofen (1 mg.kg^{1}) did not statistically modify diazepam-induced reduction of the drinking inhibition. In rats with shocks, baclofen

[1] E. COSTA, A. GUIDOTTI, C. C. MAO and A. SURIA, Life Sci. *17*, 167 (1975).

[2] P. SOUBRIE, M. H. THIEBOT, P. SIMON and J. R. BOISSIER, Life Sci., in press (1976).

[3] P. SOUBRIE, P. SIMON and J. R. BOISSIER, Experientia *32*, 359 (1976).

(2 mg.kg^{-1}) given before the 'test phase' did not statistically reduce the time spent drinking of animals treated with diazepam (4 mg.kg^{-1}) before the 'shocks-phase'.

Discussion. A reduction of the central levels of catecholamines seems to induce a memory deficit[4]. Furthermore, baclofen – as GABA-like drugs do – reduces catecholamines turn-over[5,6]. It would be tempting to relate the increase of the time spent drinking elicited by baclofen to an alteration of the memory processes induced by its activities on central catecholamines. However, under our experimental conditions, it does not seem that baclofen alters learning or memory processes but more likely impairs the transfer of the conditioned suppression from the drug to saline. This dissociation appears to be asymmetrical, since conditioned suppression transferred from water to drug.

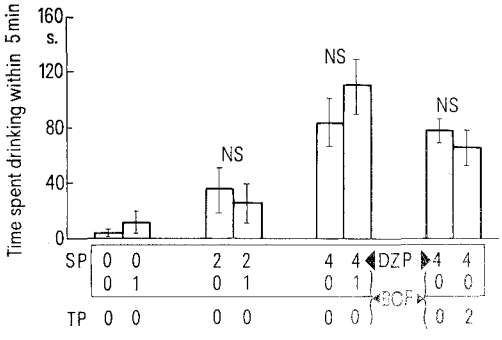

Fig. 2. 'Test phase'. Effects of baclofen given before either the 'shocks phase' (SP) or the 'test phase' (TP) on diazepam effects on the drinking inhibition. DZP, diazepam; BCF, baclofen; NS, no statistically significant difference between baclofen treated and non treated rats. Vertical bars represent SEM.

Baclofen did not modify diazepam-induced amnesia: baclofen (given before the 'shocks phase') did not potentiate the effect of diazepam on the drinking inhibition, nor (given before the 'test phase' did it elicit a reduction of the increased drinking time induced by diazepam. These data do not allow one to relate the amnesia – or an eventual dissociation of learning responsible for such an effect – induced by diazepam to those of its biochemical effects also exerted by baclofen or GABA-like drugs[5–7].

Finally, these data seem not to support a strong relationship between the GABA-enhanced receptor activity induced by benzodiazepines[1,7] and their amnesic effect. The induction of dissociation of learning seems a property of drugs – anticholinergic drugs particularly[8] – which are known to elicit in man a state of confusion. Accordingly, the results obtained with baclofen, under our experimental conditions, suggest that this drug may induce in man a state of confusion.

[4] M. E. HALL and M. MAYER, Pharmac. Biochem. Behav. *3*, 579 (1975).

[5] K. FUXE, T. HÖKFELT, L. F. AGNATI, A. LJUNGDAHL, M. PEREZ DE LA MORA and O. JOHANSSON, Abstracts 6th Int. Congress. Pharmac., Helsinki (1975), Abstr. No. 399, p. 173.

[6] E. L. NOACH, B. J. VAN ZWIETTEN-BOOT and C. F. N. VON VALKENBURG, Abstracts 6th Int. Congress Pharmac., Helsinki (1975), Abstr. No. 189, p. 89.

[7] M. PEREZ DE LA MORA, K. FUXE, L. F. AGNATI, T. HÖKFELT and A. LJUNGDAHL, Abstracts 6th Int. Congress Pharmac., Helsinki (1975), Abstr. No. 400, p. 174.

[8] B. D. BERGER and L. STEIN, Psychopharmacologia *14* ,271 (1969).

Lipoperoxide Formation in the Retina in Ocular Siderosis

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29 March 1976.

Summary. Insertion of iron nail into the vitreous cavity provoked the formation of lipoperoxide in the retina. In accord with the increase in lipoperoxide in the retina, ERG began to decrease. In vitro experiment using isolated retina, lipoperoxide was found to be increased in the presence of ferric or ferrous ions, while it was inhibited by adding antioxidants or ethylenediamine tetraacetate. From these results, direct cause of retinal degeneration in siderosis could be ascribed to the formation of lipoperoxide by iron-ions liberated from the piece of iron, resulting into the degeneration of the visual cell layers of the retina.

Inspite of many experimental and clinical studies on ocular siderosis, the mechanism of retinal degeneration in this case has never been well elucidated. Taking into account of our previous finding that the formation of lipoperoxide in the retina is a possible cause for the retinal degeneration when rabbit is exposed to high concentration of oxygen[3], as well as the reports that iron or iron compound provokes the lipoperoxide formation in the tissues or cell organelles[4–9], we reached a hypothesis that the formation of lipoperoxide could be the direct cause for the retinal degeneration in the ocular siderosis. The present paper deals with experimental data on the formation of lipoperoxide in the retina caused by an iron nail in vivo or iron-ions in vitro.

Animals used were albino rabbits weighing 2–3 kg. For in vivo experiment, one eye was used for the experiment and the other for the control. After the instillation anesthesia using 0.4% benoxinate hydrochloride, the conjunctiva and Tenon's capsule were incised for about 5 mm in length in the region of the pars plana in the upper temporal quadrant and the sclera was exposed. An iron nail ($\varnothing$ 0.8 × 8 mm) sterilized was inserted carefully into the vitreous cavity through the sclera. The conjunctiva was closed by a suture and antibiotic ointment was applied.

For ERG measurements, one or two drops of 0.4% benoxinate hydrochloride were used as a topical anesthetic. After 20 min of dark adaptation, ERG was recorded with time constant, 0.03 sec, using xenon light stimulus of 2.0 jouls. The change in ERG before and after the insertion of an iron nail was followed with time until enucleation. Amplitudes of a- and b-waves were summed and the ratio values between the experimental and control were calculated.

Lipoperoxide in the retina was determined by thiobarbituric acid (TBA) method[10]. The procedure is similar to