Animals with the highest blood pressure were mated, as well as those with the lowest levels, to obtain the first generation (S_1). Beginning from S_1 and throughout the 5 subsequent generations, selective inbreeding was continued using brother-sister mating. Statistical analysis was made by Student's *t*-test.

Results and conclusions. The systolic blood pressure of 82 rats belonging to S_6 is shown in Table II. The first set of measurements obtained in intact animals, before treatment, were significantly higher in the hypertensive (H) than in the normotensive (N) rats. Further comparison of males with females within each strain showed the B.P. of males to be significantly higher than in females. Similar sex-related differences in blood pressure were previously reported in rats with inherited hypertension[5,6]. The second set of measurements were obtained in uninephrectomized rats, 4 weeks after the initial determination and 2 days after the last injection of Doca. It is evident that a marked degree of hypertension had developed in the H strain while the N rats remained normotensive. In 49 rats, changes in B.P. were recorded at weekly intervals. As illustrated in the Figure, the H rats showed an impressive rise in B.P. at the end of the 2nd week and a further increment in the 3rd week. A moderate elevation in B.P. was also noted in the N strain, the final reading did not, however, exceed normal values.

The results are in complete agreement with the work of DAHL[2], though our breeding stock and the induction of hypertension were different. While an inherited susceptibility to hypertension was demonstrated by several workers in rats[1-3] and rabbits[7], immunity to hypertension as observed in the N colony was previously reported only by DAHL. Inherited resistance to the effects of Doca-salt may explain the failure of some rats to become hypertensive when treated with Doca, irrespective of their salt consumption[8]. It may also give a clue to one or more genetic controlled mechanisms causing hypertension. Recent studies suggest a genetic difference in adrenal biosynthetic pathways between hypertensive prone (S) and resistant (R) rats[9]. The availability of N and H rats offers new opportunities for correlating gene controlled differences with susceptibility or resistance to hypertension[10,11].

Résumé. La réponse au régime Doca-sel (D.S.) a été utilisée comme critère de sélection pour séparer, par croisement consanguin, deux colonies de rats manifestant une susceptibilité bien différente à l'hypertension artérielle. Les résultats obtenus dans la 6ème génération de nos colonies normotensive et hypertensive font l'objet de ce raport. Le rôle des facteurs héréditaires dans la susceptibilité ou la résistance à l'hypertension du type D.S. est démontrée.

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Changes in Activity and Hormonal Sensitivity of Brain Adenyl Cyclase Following Chronic Ethanol Administration

Adenosine 3′,5′-monophosphate (cyclic AMP) has been established as an intracellular mediator of the action of a number of amines and polypeptide hormones[1,2] and may well play an important role in the function of the central nervous system[3]. Previous studies in our laboratory have indicated that biochemical changes observed in the brain, following chronic ethanol administration, may involve in alteration of the function of endocrine system[4,5]. In this study, we have examined the effect of acute and chronic ethanol administration on the cyclic AMP formation in mouse cerebral cortex.

Methods. Swiss albino female mice weighing 25–28 g were treated chronically with ethanol by providing only a liquid diet for 2 weeks as previously described[4-6]. The average daily dose of ethanol was 26–33 mg/g body weight. Acutely treated mice received 4 g/kg body weight of ethanol intraperitoneally as a 20% (w/v) solution in saline. Control mice were injected with an equivalent amount of isocaloric sucrose-saline solution.

Adenyl cyclase activity in a homogenate of cerebral cortex prepared in 0.32 *M* sucrose was measured by the chromatographic separation of cyclic ^{3}H-AMP formed from ^{3}H-ATP as described by KRISHNA et al.[7]. In preliminary experiments, the measurement of UV-absorption at 260 nm following the chromatographic separation of ATP, ADP, cyclic AMP, adenine and 5′-AMP on Dowex 50 ion-exchange columns[7] was employed and confirmed that the cyclic AMP fraction taken in this experimental procedure contains only cyclic AMP. The basic incubation medium contained *tris*-HCl (pH 7.3): 40 m*M*, $MgSO_4$: 3.3 m*M*, theophylline: 10 m*M*, *tris* ATP: 2 m*M* (including 0.125 m*M* of ^{3}H-ATP (S.A.; 7.93 C/mmole) and 1-2 mg protein of enzyme preparation. Incubations were carried out for 7 min at 30 °C and terminated, after the addition of 10 µmoles of carrier cyclic AMP, by immersion in a boiling bath for 2 min. In each experiment the recovery of the carrier cyclic AMP was determined spectrophotometrically and used to correct the experimental value

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Table I. Effect of ethanol administration on cyclic AMP formation in mouse cerebral cortex

A) Homogenate	Adenyl cyclase activity (*p*mole cyclic AMP/mg protein/min ± S.D	
	Chronic[a]	Acute[b]
Control	176 ± 20 (5)	170 ± 32 (4)
EtOH treated	253 ± 22[c] (5)	183 ± 21 (4)
B) Slice	**Cyclic AMP formation (% Conversion) ± S.D.**	
	Chronic[a]	Acute[b]
Control	0.34 ± 0.06 (5)	0.29 ± 0.04 (5)
EtOH treated	0.62 ± 0.04[c] (5)	0.31 ± 0.05 (5)

[a] Ethanol-treated mice received a liquid diet containing 6% ethanol for 2 weeks. [b] Ethanol-treated mice were sacrificed 3 h following i.p. injection of 4 g/kg ethanol. [c] $p < 0.01$, compared with respective controls. Numbers in parentheses indicate the number of experiments.

Table II. Effect of in vitro addition of various drugs on cyclic AMP formation in mouse cerebral cortex of control and 2 weeks ethanol-treated mice

A) Homogenate	Adenyl cyclase activity (*p*mole cyclic AMP/mg protein/min) ± S.D.	
	Sucrose diet	EtOH diet
Control	180 ± 19 (6)	246 ± 20 (5)
+ NaF (10 m*M*)	387 ± 68[a] (5)	405 ± 59[a] (5)
B) Slice	**Cyclic AMP formation (% Conversion) ± S.D.**	
	Sucrose diet	EtOH diet
Control	0.35 ± 0.08 (5)	0.60 ± 0.02 (5)
+ DL-Norepinephrine (0.1 m*M*)	0.68 ± 0.04[a] (5)	0.65 ± 0.19 (5)
+ Histamine (0.1 m*M*)	0.56 ± 0.05[a] (5)	0.78 ± 0.07[a] (5)

[a] $p < 0.01$, compared with respective controls. Numbers in parenthesis indicate the number of experiments.

for cyclic ^{3}H-AMP. For the measurement of radioactivity, an aliquot of sample was added to 10 ml of a counting fluid containing 20% (v/v) Bio-Solv solubilizer, Formula BBS-3.

The formation of cyclic AMP in slices of the cerebral cortex was measured by a modification of the method of SHIMIZU et al.[8]. Following the pulse labelling of ATP in slices of cerebral cortex by incubation for 40 min in a medium of Krebs-Ringer-bicarbonate solution containing 2.5 μC/ml of (8-^{14}C)-adenine (S.A.: 5.45 mC/m*M*), the slices from both ethanol treated and control animals were incubated in media containing various drugs. Following 4 min incubation the reaction was stopped by the addition of 10 μmoles cyclic AMP, followed immediately by boiling for 3 min and homogenization. This homogenate was brought to near dryness by a cool air blower and the residue was suspended and centrifuged. Total radioactivity was determined and cyclic ^{14}C-AMP was separated chromatographically from other labelled nucleotides in an aliquot of the supernatant, as described by KRISHNA et al.[7]. The results were expressed as the percentage of total ^{14}C present as cyclic ^{14}C-AMP (% conversion).

Blood ethanol level[9] and protein content[10] were determined by using previously described procedures.

Results and discussion. Following continuous ethanol administration for 2 weeks, cyclic AMP formation increased in both homogenate and slice preparations of cerebral cortex (Table I). A similar increase cyclic AMP in formation was observed 1 and 3 weeks after ethanol administration. No alteration in cyclic AMP formation was detected following the acute administration of ethanol (Table I).

It is well known that the cyclic AMP formation in cerebral tissue is remarkably stimulated by the in vitro addition of various amines and fluoride[11]. Therefore we examined the effect of in vitro addition of norepinephrine and histamine on cyclic AMP formation in a slice preparation and those of fluoride were examined in a homogenate preparation by using the cerebral cortex from chronically ethanoltreated animals (Table II). Although adenyl cyclase activity in brain homogenate preparations was stimulated by fluoride in both control and ethanol-treated groups, the addition of norepinephrine to slices of the cerebral cortex stimulated cyclic AMP formation in control groups but not in ethanol-treated groups. On the other hand, the stimulatory effect of histamine on the formation of cyclic AMP in slices of the cerebral cortex was demonstrated in both control and ethanol-treated groups. Although norepinephrine content in the brain does not change following chronic ethanol ingestion[12], our present study clearly demonstrates that long-term administration of ethanol induces an increase of adenyl cyclase activity and a loss of the responsiveness of adenyl cyclase to norepinephrine in the cerebral cortex. Such a change in brain adenyl cyclase sensitivity may be an important factor in altering neurohormonal action in the brain during periods of chronic ethanol ingestion.

Zusammenfassung. Nachweis, dass durch langdauernde Äthanol-Zufuhr die Adenyl-Zyklase-Aktivität in der Grosshirnrinde von Mäusen vermehrt wird, während die fermentative Aktivität bei der akuten Zufuhr sich nicht verändert. Die Empfindlichkeit der Adenyl-Zyklase-Aktivität auf Norepinephrin im Mäusegehirn ist nach dieser Äthanol-Zufuhr deutlich herabgesetzt.

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