## Activity of Cholinergic System Enzymes in the Cochlea of Audiogenic Seizure Susceptible Mice

Some inbred strains of mice which convulse in response to a loud sonor stimulus, always show the same convulsive pattern and so provide a tool for the biological approach to epileptic behaviour. Inspite of a considerable number of investigations, biochemical mechanisms underlying audiogenic seizures are still not well defined[1-3]. A decrease of ATPase activity[4] and of oxidative phosphorylation[5], and a fall in the concentration of $\gamma$-aminobutyric acid (GABA)[6], have been reported. Protection against the seizure has been demonstrated with Krebs cycle substrates[4] or with dipropylacetate[7,8], a competitive inhibitor of GABA transaminase, which produces an increase of GABA in the central nervous system (CNS). However, the basic mechanism is unknown, even if we take into account some alterations of norepinephrine (NE), 5-hydroxytryptamine (5HT) or GABA metabolism reported[3,5].

Investigation of inborn neurochemical differences between strains sensitive or resistant to an intense auditory stimulus, may be a useful approach to clarify the mechanisms of audiogenic seizures. The sensitivity can be related to an overall increase of excitability. It was therefore of interest to investigate the pathways directly related to acoustic epilepsy. Differences at the level of the postulated peripheral receptor for the auditory stimulus, i.e. the cochlea, should be considered. Alterations may occur in filtration or amplification of the sonor stimulation. Since acethylcholinesterase (AChE; EC 3.1.1.7) activity has been detected in synaptic endings of the cochlear nucleus[9], and choline acetyltransferase (ChAc; EC 2.3.1.6) in the cochlea and the olivo-cochlear bundle[10], we have studied these two cholinergic system enzymes in the cochlea of seizure-susceptible and seizure-resistant mice of the Swiss Albino rb strain.

Choline acetyltransferase and acetylcholinesterase activities in the cochlea of audiogenic seizure susceptible and resistant mice.

| | Sensitive mice | Resistant mice |
|---|---|---|
| Choline acetyltransferase[a] | 2.84[c] ± 0.32[d] | 1.89 ± 0.40 |
| Acetylcholinesterase[b] | 555 ± 129 | 551 ± 103 |

[a] Values are expressed as μmole acetylcholine synthetized/g proteins/h.
[b] Values are expressed as mmole acetylcholine hydrolyzed/g proteins/h.
[c] Each value is the mean of 16 experiments ± S.D.
[d] $P < 0.001$.

*Materials and methods.* Susceptibility to audiogenic seizures, was tested by exposing mice, in a test chamber, to an acoustic stimulus of 100 db B, 10,000 Hz for 10 sec to 30 sec. Within 1 min mice exhibited motor patterns and convulsed as previously described[3,7].

Dissection. The cochlea of seizure-sensitive and resistant Swiss Albino rb mice was promptly excised after decapitation and frozen in dry ice. The osseus spiral lamina with the organ of Corti and spiral ganglion was then homogenized at 0–2 °C in 0.5% triton, X100 (200 μl for 1 cochlea) using Potter glass homogenizers. Assays were performed on the supernatant.

Enzyme assay. Before assay the samples were diluted with 0.05% bovine serum albumine. ChAc was measured according to the microtechnique of McCAMAN and HUNT[11] as modified by GOLDBERG, KAITA and McCAMAN[12], by following the incorporation of 1-$^{14}$C-acetate from acetyl-CoA into acetylcholine. The 1-$^{14}$C-acetylcholine formed was precipitated with potassium periodide and the precipitate counted in a Packard Scintillation counter. The enzyme activity was calculated from the known specific activity of the 1-$^{14}$C-acetyl-CoA (NEN Chemicals, 59.2 mCi/m$M$).

AChE was determined by the metod of McCAMAN, THOMEY and McCAMAN[13] based on the hydrolysis of 1-$^{14}$C-acetylcholine (NEN Chemicals, 2.43 mCi/m$M$). Unhydrolyzed substrate was precipitated as Reinecke salt. Non-specific cholinesterases were inhibited by addition of iso-octa-methylpyrophosphoramide ($1 \times 10^{-6}$ $M$). Proteins were measured by the method of LOWRY et al.[14].

*Results and discussion.* ChAc activity of the cochlea was significantly higher in mice that convulse after an intense auditory stimulus. The increase is about 50% ($p < 0.001$). In contrast, AChE activity was similar in the cochlea of both strains (Table).

Specific sensitivity of the peripheral auditory pathway has to be considered in the pathogenesis of audiogenic seizures. Thus, FULLER and COLLINS[15-17] demonstrated the unilateral nature of the site of sensitization by exposing seizure-resistant SJL/J mice to an intense sound stimulus with one ear open. Convulsions, in this case, appear only after a new stimulus of the single ear stimulated before. Thus, the site of sensitization appears to be either in the ear or in the part of the auditory pathway receiving impulses only from one side.

Therefore the responsible area for the seizure susceptibility could be investigated in the region of the first relay of the peripheric auditory pathway, namely the spiral ganglion.

Higher levels of ACh in the first acoustic relay in seizure-sensitive mice may produce stronger excitatory


[1] M.R.A. CHANGE, in *Psychophysiologie, Neuropharmacologie et Biochimie de la Crise Audiogène* (Ed. R. G. BUSNEL; Editions du C.N.R.S., Paris 1963), p. 15.
[2] L.V. KRUSHINSKI, in *Psychophysiologie, Neuropharmacologie et Biochimie de la Crise Audiogène* (Ed. R. G. BUSNEL; Editions du C.N.R.S., Paris 1963) p. 71.
[3] A. LEHMANN, Thèse d'Etat de Doctorat ès-Sciences Naturelles, Université de Paris, 1964.
[4] B. E. GINSBURG and D. S. MILLER, in *Psychophysiologie, Neuropharmacologie et Biochimie de la Crise Audiogène* (Ed. R. G. BUSNEL; Editions du C.N.R.S., Paris) p. 217.
[5] K. SCHLESINGER and J. GRIEK, in *Contributions to Behavior Genetic Analysis* (Eds. G. LINDZEY and D. D. THIESSEN; Appleton Century Crafts, New York 1970) p. 219.
[6] E. ROBERTS, M. ROTHSTEIN and C.F. BAXTER, Proc. Soc. exp. Biol Med. *97*, 796 (1958).
[7] S. SIMLER, L. CIESIELSKI, M. MAÎTRE, H. RANDRIANARISOA and P. MANDEL, Biochem. Pharmac., in press.
[8] S. SIMLER, A. LEHMANN and H. RANDRIANARISOA, C. r. Soc. Biol., Paris *165*, 1147 (1971).
[9] D. M. McDONALD and G. L. RASMUSSEN, Brain Res. *28*, 1 (1971).
[10] A. JASSER and P. S. GUTH, J. Neurochem. *20*, 45 (1972).
[11] R. E. McCAMAN and J. M. HUNT, J. Neurochem. *12*, 253 (1965).
[12] A. M. GOLDBERG, A. A. KAITA and R. E. McCAMAN, J. Neurochem. *16*, 823 (1969).
[13] M. W. McCAMAN, L. R. THOMEY and R. E. McCAMAN, Life Sci. *7*, 253 (1968).
[14] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[15] J. L. FULLER and R. L. COLLINS, Science *162*, 1295 (1968).
[16] R. L. COLLINS, Science *167*, 1010 (1970).
[17] R. L. COLLINS and R. WARD, Nature, Loud. *226*, 1062 (1970).

impulses which spread towards the CNS leading to 'epileptic' convulsions. This postulate is in agreement with KRUSHINSKY's[2] hypothesis which suggests that the development of the convulsive crisis is primarily dependent on low brain stem structures. The distribution of ChAc and AChE seems genetically independent.

*Résumé.* Le système cholinergique est étudié au niveau de la cochlée chez des souris se distinguant par leur sensibilité à l'épilepsie acoustique. Une activité accrue de l'enzyme de synthèse de l'acétylcholine a pu être mise en évidence dans la souche génétiquement sensible à la crise audiogène.

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## Extinction of the Vasodilator Component of the Defence Reaction in the Cat

The somato-autonomic relationship is perhaps expressed in the most dramatic manner in emergency situations such as aggression or flight. In these situations the somato-autonomic link is obviously vital for survival, since autonomic adjustments play an important role in enabling the performance of the behavioural task. The cardiovascular system has an essential role to play in these adjustments and its ability to respond selectively and adequately to signals from the environment is particularly important in flight or aggression.

It has been established that a typical feline, threatening posture with vocalization can be elicited by electrical stimulation in well-defined areas of the brainstem and amygdala of cats, and that this behavioural reaction is accompanied by a pattern of cardiovascular adjustment whereby the cardiac output is increased, the blood flow from the skin and mesenteric vascular beds being redirected to those of skeletal muscle[1,2]. In this cardiovascular response active vasodilatation in the skeletal muscles plays an important role and it has been shown that in cats it is brought about largely by activation of the cholinergic sympathetic nerve fibres, since injections of atropine greatly reduce it[1,3]. Moreover, a very similar cardiovascular reaction including cholinergic muscle vasodilatation occurs in animals when they are startled by a sudden and new stimulus[3]. With repetition of the stimulus the response subsides. The cholinergic muscle vasodilatation appears also as a response to noxious stimulation, and this vascular reaction has been easily conditioned when an auditory stimulus preceded the noxious stimulation[3-5]. The startle reaction to novelty ('unknown stimulus') and the reaction to noxious stimuli appear to be closely related to flight or aggression[3,6,7]. Various species respond with 'alarm', flight or display of a threatening posture to definite visual or auditory stimuli. Some cats when confronted by a dog often display a threatening posture similar to that elicited by stimulation in specific areas in the brainstem or amygdala. We have chosen the 'naturally' elicited threatening reaction in cats confronted by a dog to investigate the extent and evolution of cardiovascular involvement.

ADAMS et al.[8] have shown that cats responding to threatened aggression by another cat may do so without the cardiovascular changes so characteristic of the defence reaction elicited by brainstem stimulation. In particular, they found that the cholinergic muscle vasodilatation may be absent. It is possible that in these experiments the vasodilator response had already been extinguished before the measurements were made; this question has, therefore, been specifically examined.

In our experiments on cats, observations of behaviour were correlated with measurements of arterial blood pressure, external iliac blood flow, heart rate, electromyographic activity in the hindlimb, and respiratory rate. The external iliac blood flow was monitored using an MBI flow meter with an electromagnetic probe (Micron) implanted above the deep femoral branch which was ligated. The blood pressure was monitored via transparent vinyl cannula inserted into the aorta through the common carotid artery. The conductance (flow/pressure) in the external iliac artery was recorded continuously, with the use of an electronic divider (Analog 4 Quadrant Multiplier AD 426 in the feedback of an AD 401 operational amplifier). The cat was in a box facing a chicken wire or glass

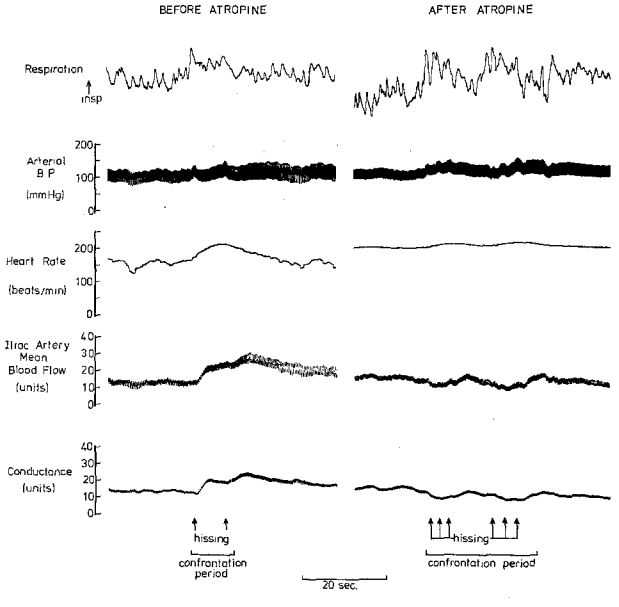


Fig. 1. Cardiovascular concomitants of the threatening response of a cat when confronted with a dog. The vasodilator component is blocked by an intra-arterial injection of atropine sulphate (0.2 mg/kg).

1 V. C. ABRAHAMS, S. M. HILTON and A. W. ZBROŻYNA, J. Physiol., Lond. *154*, 491 (1960).
2 A. W. ZBROŻYNA, *The Neurobiology of the Amygdala* (Ed. ELEPHTERIOU; Plenum Press, New York 1971), p. 597.
3 V. C. ABRAHAMS, S. M. HILTON and A. W. ZBROŻYNA, J. Physiol., Lond. *171*, 189 (1964).
4 P. BÖLME and J. NOVOTNY, Acta physiol. scand. *77*, 58 (1969).
5 C. J. SUTHERLAND and A. W. ZBROŻYNA, J. Physiol., Lond. *218*, 83 P (1971).
6 T. C. SCHNEIRLA, Adv. Study Behav. *1*, 1 (1965).
7 R. URLICH and B. SYMANNEK, in *Aggressive Behaviour* (Ed. GARATTINI and SIGG; Proc. Int. Symp. Biol. Aggr. Beh., Milan 1968 (Elsevier, Amsterdam 1969), p. 59.
8 D. B. ADAMS, G. BACCELLI, G. MANCIA and A. ZANCHETTI, J. Physiol., Lond. *212*, 321 (1971).