Taux d'amines cérébrales d'une souche de souris «tremblantes»

Les souris tremblantes («quaking») sont des mutants provenant d'une lignée (DBA) de souris noires[1]. Cliniquement, elles présentent des crises convulsives; la fréquence des crises varie d'un animal à l'autre, leur survenue est soit apparemment spontanée, soit provoquée par un stimulus sensoriel comme par exemple la simple manipulation de l'animal.

A l'examen histologique, les souris tremblantes se distinguent des souris non atteintes de la même lignée par un important déficit en myéline au niveau du système nerveux central[1]. Parallèlement, on observe des anomalies qualitatives et quantitatives des lipides cérébraux[2,3].

Nous avons par ailleurs décrit et analysé le comportement de ces souris[4]. En outre, nous avons recherché si aux modifications biochimiques déjà décrites s'associaient des variations des concentrations cérébrales des principales amines biogènes: noradrénaline, dopamine, 5-hydroxytryptamine, acétylcholine et histamine. Enfin, nous avons recherché un éventuel rapport entre ces variations et la fréquence des crises observées.

Matériel et méthodes. Les souris ont été triées au 30e jour et réparties en 2 groupes, tremblantes ou témoins, selon qu'elles présentaient ou non des tremblements et des crises tonico-cloniques après stimulation tactile. Elles ont été sacrifiées entre le 40e et 50e jour, les cerveaux étant immédiatement prélevés, refroidis à −4 °C, puis pesés. Les dosages suivants ont été effectués: Sur un cerveau, dosage simultané de la noradrénaline[5], la dopamine[6] et la 5-hydroxytryptamine[7] (30 souris). Sur un cerveau, dosage de l'histamine[8] (15 souris). Sur un groupe de 3 cerveaux, dosage de l'acétylcholine[9] (30 souris). Dans le premier groupe, avant sacrifice, on compte le nombre de crises spontanées de chaque souris pendant 10 min.

Résultats. Les résultats sont indiqués sur le Tableau. Les concentrations cérébrales de noradrénaline, de dopamine et d'acétylcholine des souris tremblantes sont significativement augmentées, alors que les poids des cerveaux sont significativement diminués. Il n'existe aucune corrélation entre l'élévation de ces concentrations et le nombre de crises observées. Les autres amines, histamine et 5-hydroxytryptamine, ne sont pas modifiées. Les poids corporels des deux groupes d'animaux ne diffèrent pas significativement.

Discussion. Chez ces souris de même âge, les poids des cerveaux des souris tremblantes sont plus faibles que ceux des témoins. Ce fait est à rapprocher des anomalies constatées lors de l'étude histologique, absence de myéline, diminution globale des lipides cérébraux. Les augmentations observées de l'acétylcholine et des catécholamines cérébrales ne sont pas interprétables actuellement; on peut cependant supposer qu'elles sont soit la cause, soit la conséquence des lésions observées. Une étude cinétique de leurs vitesses de renouvellement permettra éventuellement de préciser leur importance.

Taux d'amines et poids cérébraux M ± S_{ra} des souris DBA, tremblantes ou témoins

	Souris «tremblantes»	Souris normales (de même souche)
5-OH tryptamine (µg/g)	0,504 ± 0,021	0,439 ± 0,036
Noradrénaline (µg/g)	0,449 ± 0,009[a]	0,365 ± 0,007
Dopamine (µg/g)	1,320 ± 0,033[a]	0,974 ± 0,031
Acétylcholine (µg/g)	2,790 ± 0,218[b]	2,090 ± 0,136
Histamine (ng/g)	45,8 ± 4,5	41,5 ± 3,7
Poids du cerveau (g)	0,393 ± 0,005[a]	0,432 ± 0,002

[a] $p < 0{,}001$; [b] $p < 0{,}02$.

Summary. In the 'quaking' mouse, an autosomal recessive mutant characterized by a deficiency of central nervous system myelin, the brain levels of 5-hydroxytryptamine and histamine are unchanged and the brain levels of norepinephrine, dopamine and acetylcholine are significantly increased.

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Unité de Recherches de Neuropsychopharmacologie INSERM, 2, rue d'Alésia, F-75 Paris 14e (France), 2 septembre 1970.

[1] R. L. SIDMAN, M. C. GREEN et S. H. APPEL, *Catalog of the Neurological Mutants of the Mouse* (Harvard University Press, 1965).
[2] N. A. BAUMANN, C. M. JACQUE, S. A. POLLET et M. L. HARPIN, Europ. J. Biochem. *4*, 340 (1968).
[3] C. M. JACQUE, M. L. HARPIN et N. A. BAUMANN, Europ. J. Biochem. *11*, 218 (1969).
[4] M. A. GOUJET, P. SIMON et I. R. BOISSIER, Experientia, soumis pour publication.
[5] U. S. VAN EULER et F. LISHAJKO, Acta physiol. scand. *51*, 348 (1961).
[6] R. K. HINESTLEY, J. A. NORTON et M. H. J. APRISON, Psychiat. Res. *6*, 143 (1968).
[7] R. P. MAICKEL, R. H. COX JR., J. SAILLANT et F. P. MILLER, Intern. J. Neuropharmac. *7*, 275 (1968).
[8] J. R. BOISSIER, M. GUERNET, J. P. TILLEMENT, I. BLANCO et M. BLANCO, C. r. Acad. Sci., Paris *268*, 1448 (1969).
[9] B. N. SMALLMAN et R. W. FISCHER, Can. J. Biochem. Physiol. *36*, 575 (1958).

An Investigation of Na Efflux from the Toad Oocyte Using Cold Shock as a Technique

There is evidence that ethacrynic acid produces a large fall in Na efflux from the oocyte of the toad, *Bufo bufo*[1]. However, this effect develops somewhat slowly so that estimation of its size by extrapolating the last points on the curve to the time of application of the inhibitor results in an underestimation. The same is true when the slopes of the curve before and after applying ethacrynic acid are compared. The purpose of this paper is to report the results of experiments with chilled oocytes and show that one can arrive at an estimate of the magnitude of the inhibition simply by rewarming the oocyte in the presence of ethacrynic acid. This paper also brings forward evidence which shows that the application of ouabain in a warm environment to oocytes previously chilled and poisoned with ethacrynic acid stimulates the residual efflux.

Immature oocytes measuring 600–1200 μm in diameter were dissected from healthy female specimens of *Bufo bufo*. They were then loaded with ^{24}NaCl as described by DICK and LEA[2]. All transfers were done with a braking pipette[3]. Counting of ^{24}Na activity of the oocyte and effluent was carried out as described by DICK and LEA[2] and BITTAR, DICK and FRY[1]. Chilling to 0 °C was effected by placing the oocytes on the one inch phosphor bases in a metal chamber surrounded by ice and meltwater, and chilling to 4 °C by placing the chamber in the bath of a cooler manufactured by Grant Instruments (Cambridge) Ltd. Both Ringer's solution and the phosphor bases were cooled before use.

Ethacrynic acid was a gift from Dr. J. E. BAER of the Merck Institute for Therapeutic Research, West Point, Pennsylvania, USA. Ouabain was obtained from British Drug Houses Ltd., Poole, Dorset.

The effect of low temperature on Na efflux. Figure 1 is that of a typical control experiment showing that an environmental temperature of 0 °C reduces the loss of ^{24}Na to values approaching passive levels. Also shown is that rewarming leads to complete restoration of the efflux, a result which agrees with that of HODGKIN and KEYNES[4] using squid giant axons.

As illustrated by Figure 2 an environmental temperature of 4 °C causes a substantial fall in Na efflux, leaving an efflux which is interrupted by a temperature of zero.

The effect of ethacrynic acid on rewarmed oocytes. The application of $10^{-3}M$ ethacrynic acid to chilled oocytes, followed some 3 h later by rewarming is found to almost completely prevent the restoration of Na efflux to control values. This result is illustrated by Figure 3. It will be seen that the magnitude of the inhibition caused by ethacrynic acid is easily computed on the basis of the change in efflux.

The effect of ouabain on rewarmed oocytes poisoned with ethacrynic acid. The application of $10^{-4}M$ ouabain to rewarmed oocytes, following treatment with $10^{-3}M$ ethacrynic acid at 0 °C and room temperature for more than 6 h, promptly raises the Na efflux, as illustrated by Figure 4. This is in striking contrast to the inhibiting

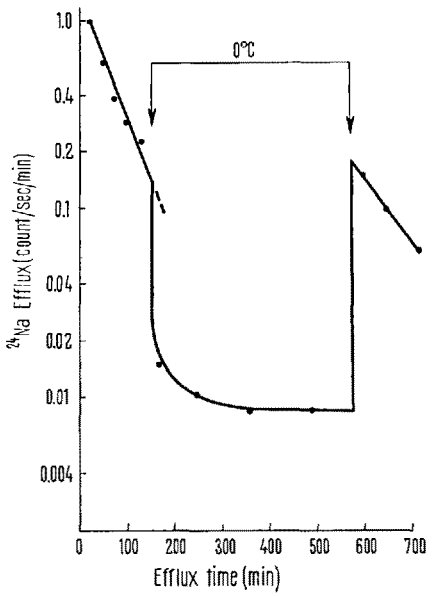

Fig. 1. Semi-logarithmic plot showing the effect on radio-sodium efflux of an environmental temperature of 0 °C. Horizontal arrows indicate duration of cooling (room temperature ca. 19 °C).

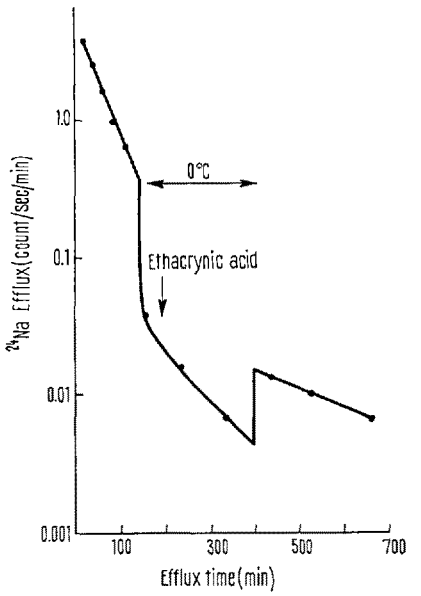

Fig. 3. The inhibiting effect of $10^{-3}M$ ethacrynic acid on Na efflux following rewarming of a chilled oocyte. Vertical arrow indicates time at which inhibitor was applied.

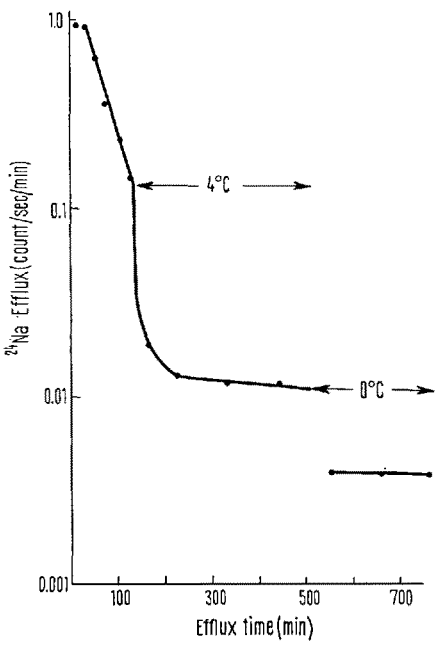

Fig. 2. The effect on Na efflux of an environmental temperature of 4 °C and 0 °C.

[1] E. E. BITTAR, D. A. T. DICK and D. J. FRY, J. Physiol., Lond. *196*, 693 (1968).
[2] D. A. T. DICK and E. J. A. LEA, J. Physiol., Lond. *174*, 55 (1964).
[3] H. HOLTER, C. r. Trav. Lab. Carlsberg, sér. chim. *24*, 399 (1943).
[4] A. L. HODGKIN and R. D. KEYNES, J. Physiol. *128*, 28 (1955).

effect of ouabain on unchilled oocytes pretreated with ethacrynic acid[1].

Abolition of active Na^+ transport by an environmental temperature of 0 °C is shown to be rapid and reversible. These features are suggestive of an enzymic process or interruption of the energy supply to the Na pump, or both. The observation that a temperature of 4 °C fails to almost entirely stop Na efflux is in keeping with the idea that cold of this degree neither completely interrupts the Na^+-K^+-ATPase[5,6] nor the phosphorylation of ADP[7].

The Na efflux is drastically reduced by ethacrynic acid when chilled oocytes are treated sufficiently long with the inhibitor before rewarming. The size of the effect, roughly 90% in the majority of cases, is comparable to that produced by ethacrynic acid when unchilled oocytes are treated for more than 3 h[1]. It is thus evident that the use of cold shock is one simple way by which information obtained at room temperature can be verified.

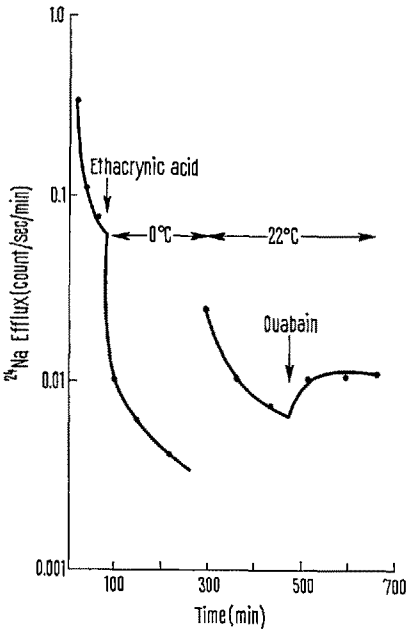

Fig. 4. The stimulating effect of $10^{-4}M$ ouabain on the residual efflux following rewarming of a chilled oocyte poisoned with ethacrynic acid. Second vertical arrow indicates time at which ouabain was applied.

A puzzling feature of the experiments with ouabain is that the inhibitor stimulates the residual efflux from oocytes poisoned with ethacrynic acid before rewarming. One may speculate that ouabain like oligomycin[8] has the power to mobilize the sequestered fraction of Na in the oocyte. Evidence favouring such an explanation comes from unpublished experiments showing that the Na efflux into K-free Ringer is frequently stimulated by ethacrynic acid or ouabain. The kinetics of Na efflux under these experimental conditions clearly indicate that the liberation of the sequestered Na caused by ouabain and ethacrynic acid leads to partial saturation of the pump[9].

Zusammenfassung. Die infolge der verzögerten Wirkung von Ethacrynsäure nur näherungsweise erfassbare Hemmung der Abgabe von Na^+ durch Oozyten der Kröte (*Bufo bufo*) lässt sich exakter bestimmen, wenn die chemische mit einer Temperaturbehandlung kombiniert wird.

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Department of Human Anatomy, University of Oxford, Oxford (England), 7 September 1970.

[5] N. GRUENER and Y. AVI-DOR, Biochem. J. *100*, 762 (1966).
[6] M. W. SMITH, Biochem. J. *105*, 65 (1967).
[7] H. W. HELDT and M. KLINGENBERG, Europ. J. Biochem. *4*, 1 (1968).
[8] E. E. BITTAR, Life Sciences *9*, 773 (1970).
[9] Acknowledgments. This work was carried out in collaboration with Dr. D. J. FRY and was supported by the Wellcome Trust.
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Effect of Heavy Physical Training on the Catecholamine Content of the Heart and Adrenals of the Guinea-Pig

In recent studies[1,2], a significant reduction in heart catecholamines after prolonged physical training has been described. In these studies a comparatively low training load was used, furthermore the animals were exercised only 3 times a week. In the present study the effect of a comparatively intense daily training program on the catecholamine contents of the heart and the adrenals was studied.

Materials and methods. Young male guinea-pigs were used. The animals were trained on a treadmill with gradually increased intensity throughout 5 months. The initial training consisted of running for 60 min at a speed of 22 m/min. In the last month, the animals had to run for 80 min at a speed of 37 m/min and a subsequent period of 15 min at a speed of 57 m/min. After 4 days rest, the animals were sacrificed by a blow on the head. The heart and the adrenals were taken out, cleaned and weighed. The noradrenaline content of the heart was extracted, as previously described[3], and determined according to CHANG[4]. The adrenal catecholamines were extracted[5] and determined according to EULER and LISHAJKO[6]. The catecholamine values are given as μg free base.

Results. The results are shown in the Table. The trained animals show a significant increase in heart ratio.

[1] C. DE SCHRYVER, P. DE HERDT and J. LAMMERANT, Nature, Lond. *214*, 907 (1967).
[2] C. DE SCHRYVER, J. MERTENS-STRYTHAGEN, I. BECSEI and J. LAMMERANT, Am. J. Physiol. *217*, 1589 (1969).
[3] N. O. SJÖSTRAND and G. SWEDIN, Acta physiol. scand. *72*, 370 (1968).
[4] C. C. CHANG, Int. J. Neuropharmac. *3*, 643 (1964).
[5] T. BRUNDIN, Acta physiol. scand., *70*, Suppl. 290 (1966).
[6] U. S. VON EULER and F. LISHAJKO, Acta physiol. scand. *58*, 1 (1963).