Action of New β-Blocking Agents on the Submaxillary Gland of the Rat

In the submaxillary gland of the rat isoprenaline causes secretion as shown already by SELYE, VEILLEUX and CANTIN[1]. Secretory effects of adrenaline, noradrenaline and sympathetic stimulation can be greatly reduced but usually not abolished by α-blocking compounds[2,3]. These observations suggest that some β-receptors are present in the gland. The responses persisting after α-blockers can be further diminished but not completely annulled by pronethalol[3]. In the present investigation some more recent β-blockers were tested on the submaxillary gland of the rat.

The rats were anaesthetized with chloralose (80–100 mg/kg into a cannula in the femoral vein after induction with ether). Tracheal cannula was inserted, and the submaxillary ducts were exposed in the neck and cannulated using fine glass cannulae. In many experiments the chorda-lingual nerve was cut on one side 2–4 weeks prior to the acute experiment in order to sensitize the gland and thus increase the secretory responses. The experiments often lasted for as much as 8 h and pentobarbitone (20 mg/kg) was then supplied i.p. at intervals to ascertain a sufficiently deep anaesthesia. Secretory drugs (isoprenaline and adrenaline 10 μg/kg; methacholine 5 μg/kg) were injected i.v. every 5 min. The cervical sympathetic trunk was stimulated electrically during periods of 1–2 min. The following blocking agents were studied: dibenzyline, dihydroergotamine, propranolol, 1-INPEA (D(−)-1-(nitrophenyl)-2-isopropylaminoethanol hydrochloride) and metoxamine.

After dibenzyline (0.4 mg/kg) and dihydroergotamine (0.2–0.4 mg/kg) the responses to isoprenaline and methacholine were unaffected and those to adrenaline and sympathetic stimulation very much reduced. Propranolol (2 mg/kg) was found completely to abolish the secretory responses to adrenaline and sympathetic stimulation remaining after the injection of α-blockers in most glands. The effect of isoprenaline was likewise abolished, that of methacholine was not changed. The blocking action of propranolol was found to last for several hours. INPEA had a similar effect as propranolol, but complete abolishment of the secretory responses to adrenaline or sympathetic stimulation (after α-blockers) or to isoprenaline was often difficult to achieve owing to the arousal effect of the drug in high dosage. In some glands the responses were completely suppressed by 25–50 mg INPEA/kg, but in other animals doses of this order caused arousal which was often accompanied by salivary secretion. A β-blocking action on the heart, on blood vessels and smooth muscles has been attributed to metoxamine[4]. In the present experiments no β-blocking effect of this drug could be observed. Doses as high as 10 mg/kg were tried; they caused a long-lasting slow flow of saliva.

The experiments support previous conclusions that the submaxillary gland of the rat is richly supplied with α-receptors for catecholamines and in addition with some β-receptors. They show that propranolol and INPEA are more efficient β-blocking drugs on this preparation than pronethalol[5].

Zusammenfassung. An narkotisierten Ratten wird gezeigt, dass die Submaxillarisdrüsen sowohl mit α- als auch mit β-Rezeptoren für Katecholamine versehen sind. Propranolol und 1-INPEA (D(−)-1-(p-Nitrophenyl)-2-isopropylaminoethanol hydrochloride) ergeben eine wirkungsvolle Blockade der β-Rezeptoren der Drüse.

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[1] H. SELYE, R. VEILLEUX and M. CANTIN, Science *133*, 44 (1961).
[2] N. EMMELIN and B. C. R. STRÖMBLAD, Experientia *19*, 104 (1963).
[3] N. EMMELIN, J. HOLMBERG and P. OHLIN, Br. J. Pharmac. Chemother. *25*, 134 (1965).
[4] S. M. M. KARIM, Br. J. Pharmac. Chemother. *24*, 365 (1965).
[5] Propranolol (Inderal) was kindly supplied by A. B. SCANMEDA, Gothenburg, and INPEA by A. B. DRACO, Lund.

Ethanol Inhibition of Audiogenic Stress Induced Cardiac Hypertrophy

Numerous investigations have presented evidence that ethanol when chronically ingested in excessive amounts is detrimental to both the socioeconomic and physiologic status of the individual[1–8].

Animal studies have investigated the interactions of ethanol with a number of the compounds (serotonin, norepinephrine, dopamine and gamma aminobutyric acid) found in the central nervous system that are postulated to play a role in its function[4–6].

Ethanol has been further demonstrated to possess some of the general capabilities (properties) of a stressor of the type outlined by SELYE 1950)[7–10]. On the other hand, ethanol in low concentration can manifest attenuative or protective actions against some types of body stress and improve certain types of mental performance[11–13].

Previous studies by the present authors had indicated that chronic 'emotional' (audiogenic) stress in the rat produced a number of biochemical, anatomical and fetal alterations[14–16]. It was also demonstrated that the ad lib ingestion of 10% ethanol by the rats during the stress periods partially blocked the biochemical responses[17]. One of the most interesting observations was the fact that although this type of stress (audiogenic) resulted in a slight decrease in body, adrenal and kidney weight, the heart showed significant hypertrophy (increase in weight). Therefore, it became of interest to attempt to inhibit these anatomic alterations, and the cardiac hypertrophy in particular, by as physiologic a means as possible. Ethanol was chosen as an adequate pharmacologic prototype compound capable of being accepted easily by the experimental animals and assimilated readily by their metabolic pathways.

Virgin female albino rats (Sprague-Dawley) were used throughout the present investigation. They were maintained on Purina Rat Chow, lettuce, bread, potato and water for 1 month before use.

The experimental animals were subjected to the auditory stress by placing them in a specially constructed, sheet metal chamber measuring 40 · 52 · 48 inches. Six

8-inch Utah speakers were mounted on 2 sides of the chamber. The output of a Grason-Stadler Noise Generator (Model 901A), and an RCA Audio Generator (Model WA-44C) was directed into the interior of the chamber by the speaker system. Ten additional noise generators (bells, gongs, buzzers, alarms etc.) were mounted on the interior walls of the experimental chamber and connected to programmed timing devices which allowed the sequence of noises to be set so that the total effective auditory stress time was 10% (6 min of each hour of the day) with 90% (54 min of each hour of the day) of the experimental period containing only the ambient background noise level of 64 decibels (db). The average sound level produced in the stress chamber by the noise generator was 83 db (range 73–93) with a frequency range of 20 c/sec to 25,000 c/sec. The output of all sound generating equipment was continually recorded with a General Radio Noise Meter type 1551A.

At the end of the experimental period (2 weeks) the animals were removed from the chamber, quickly decapitated with a Harvard Decapitor Model 130M, bled, organs removed and weighed on a Mettler Analytical Balance type H16 (capacity 80 g). Control groups, exposed only to the ambient noise level of 64 db in the animal room, were sacrificed and weighed in the same manner at this time.

Experimental and control groups (60 animals each) drinking alcohol were given the solution 48 h prior to the start of the experimental period to allow them to adjust to the new drinking solution. The ethanol was prepared by dilution of absolute ethanol with distilled water to make a final concentration of 10%.

The Table presents the data in such a manner that the effect of the 2 interacting variables, i.e. ethanol ingestion and audiogenic stress, on organ weight can be considered either alone or in combination for evaluating their respective contributions to the overall results. Note that ingestion of ethanol for 2 weeks under control conditions resulted in a small (4.3%) decrease in body weight; audiogenic stress alone also produced a small decrease (2.6%); a combination of stressing the animals while they were ingesting ethanol also produced a decrease in body weight (2.7%). The relationships with reference to the adrenals are much the same. Kidney weights in animals drinking ethanol under control conditions were increased (8.8%) but under all other comparable combinations of factors the data were essentially similar to that for body and adrenal weights. By contrast, the heart manifested a weight increase response to ingestion of ethanol (3.4%) under control conditions, and to stress (10.2%) while the animal was drinking water during this period. The effect on the heart produced by drinking ethanol while under stress conditions was noted to be a decrease (12.8%) in the weight of the organ, essentially the same type response shown by the other body parameters under similar conditions.

Several reports indicate that various types of stress, e.g. swimming, are capable of reducing the depressant or intoxicating effect of ethanol on the central nervous system[18,19]. The present study has demonstrated that ethanol ingestion under the control conditions of the

[1] E. M. Jellinek, Can. med. Ass. J. *83*, 1341 (1960).
[2] M. Hebbelinck, Archs int. Pharmacodyn. Thér. *120*, 402 (1959).
[3] M. J. G. Lynch and P. Hillis, Archs Path. *69*, 342 (1960).
[4] D. Gursey and R. E. Olson, Proc. Soc. exp. Biol. Med. *104*, 280 (1960).
[5] G. R. Pscheidt, B. Issekutz and H. E. Himwich, Jl Stud. Alcohol *22*, 550 (1961).
[6] A. H. Feldstein, H. Hoagland, K. Wong and H. Freeman, Q. Jl Stud. Alcohol *25*, 218 (1964).
[7] J. J. Smith, J. clin. Endocr. Metab. *11*, 792 (1951).
[8] G. A. Santisteban, Q. Jl Stud. Alcohol *22*, 1 (1961).
[9] E. S. Perman, Acta physiol. scand. *48*, 323 (1960).
[10] H. Selye, *The Physiology and Pathology of Exposure to Stress* (Acta Inc. Med. Publ., Montreal 1950).
[11] L. A. Greenberg and D. Lester, Q. Jl Stud. Alcohol *14*, 385 (1953).
[12] R. Adamson and R. Black, J. Comp. physiol. Psychol. *52*,734 (1959).
[13] J. A. Carpenter, O. K. Moore, C. R. Snyder and E. S. Lisansky, Q. Jl Stud. Alcohol *22*, 183 (1961).
[14] W. F. Geber, T. A. Anderson and B. Van Dyne, Growth *30*, 87 (1966).
[15] W. F. Geber and T. A. Anderson, Archs envir. Hlth *12*, 751 (1966).
[16] W. F. Geber, J. Embryol. exp. Morph. *16*, 1 (1966).
[17] W. F. Geber, T. A. Anderson and B. Van Dyne, Expl Med Surg. *24*, 25 (1966).
[18] A. Leikola, Q. Jl Stud. Alcohol *23*, 369 (1962).
[19] D. M. Woodbury, Pharm. Rev. *10*, 275 (1958).

Effect of ethanol ingestion on organ weight alteration due to chronic audiogenic stress

	Absolute weight (mg)		Relative weight (mg/100 g body weight)	
	Control	Experimental	Control	Experimental
Body weight				
Water	$268 \pm 5 \cdot 10^3$	$261 \pm 6 \cdot 10^3$	–	–
Ethanol	$256 \pm 5 \cdot 10^3$	$249 \pm 4 \cdot 10^3$	–	–
Adrenal[a]				
Water	37 ± 0.3	34 ± 0.2	13.8 ± 0.05	13.0 ± 0.1^d
Ethanol	33 ± 0.1	31 ± 0.1	12.9 ± 0.1^e	12.5 ± 0.1^e
Heart[b]				
Water	828 ± 5	888 ± 6	308.5 ± 1.6	340.0 ± 1.1^d
Ethanol	816 ± 4	738 ± 5	319.0 ± 1.1^e	296.0 ± 1.3^{de}
Kidney[c]				
Water	915 ± 9	880 ± 5	342.0 ± 1.5	337.0 ± 1.2n.s.
Ethanol	950 ± 5	842 ± 4	372.0 ± 1.1^e	338.0 ± 1.6^d

All values are means ± S.E. [a] Both adrenals averaged initially. [b] Ventricles only. [c] Both kidneys averaged initially. [d] Significant change from control ($P = < 0.01$). [e] Significant difference between water and ethanol ($P = < 0.01$). n.s., not significant.

experiment was capable of producing an increase in the weight of both the kidney and heart. In addition, the exposure of the animals to chronic, medium intensity (83 db) audiogenic stress produced a significant increase in the weight of the heart. A number of studies have clearly shown that noise is a potent stressor agent[20,21]. Ethanol has also been suggested as another type of stressor[8,9]. Certainly in the present investigation, as far as the heart and kidneys are concerned, this appeared to hold true. Yet, as the data clearly indicate, the ingestion of ethanol was an effective means of blocking or inhibiting the increase in heart weight due to audiogenic stress alone. In addition, ethanol's own weight increasing influence on the heart was nullified. The combination of ethanol and audiogenic stress also resulted in elimination of the individual effects of the 2 factors on the weight of the kidney. Thus, ethanol in the concentration employed in the present study was apparently functioning as an antistressor agent in the presence of another stressor with different characteristics[22].

Zusammenfassung. Gruppen weiblicher jungfräulicher Albinoratten wurden 2 Wochen lang periodischem Lärm- und Geräuschhintergrund ausgesetzt, während welcher Zeit sie entweder Leitungswasser oder Äthanol trinken durften. Kontrollgruppen wurden bei Abwesenheit des Lärmhintergrunds geprüft. Herzhypertrophie konnte in den Kontrollgruppen mit Äthanol und bei den Ratten (mit Lärmhintergrund) beobachtet werden. Nach Behandlung mit Äthanol war die Herzhypertrophie nicht mehr vorhanden.

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[20] E. J. FARRIS, E. H. YEAKEL and H. S. MEDOFF, Am. J. Physiol. *144*, 331 (1945).

[21] A. M. SACKLER, A. S. WELTMAN and P. JURTSHUK, Aerospace Med. *31*, 749 (1960).

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Conduction Block in Capsula Interna Fibres Caused by Striatal Spreading Depression in Rats

Depolarization of neural elements[1,2] with the consequent release of potassium is a necessary prerequisite of the self-maintained spreading of LEAO's[3] spreading depression (SD). On the contrary, SD does not penetrate across layers of white matter[4], probably because the myelinated axons do not release enough potassium ions to maintain the spreading of the process. It remained an open question whether myelinated fibres in the depressed region are passively depolarized or whether they continue conducting. The present paper shows that even gross bundles of myelinated fibres may be blocked by SD invading the adjacent grey matter.

Method. Experiments were performed on 12 albino rats aged 3 months. Tracheotomy and trephine openings were made under light ether anaesthesia. The animals were then immobilized with D-tubocurarine (2 mg/kg) and placed into a stereotaxic apparatus. They were maintained under artificial respiration (open system 60/min). Striatal spreading depression (StSD) was elicited by microinjection of 0.2–0.3 μl 25% KCl into the head of the caudate nucleus through the injector part of an electrode-cannula assembly. The glass capillary (300 μ outside diameter) connected to a calomel cell electrode, was used to lead off the slow potential changes from a point 1.5 mm remote from the cannula orifice. The stereotaxic coordinates of the injection and recording points were AP – 2.0, L 2.0, V 5.0 and AP 0.0, L 2.5, V 4.5 respectively (according to the atlas by FIFKOVÁ and MARŠALA[5]). Cortical EEG and steady potentials were picked up with wick calomel cell electrodes applied onto the brain surface exposed by trephine openings (5 mm in diameter) above the somatosensory or visual cortex. Somatosensory evoked responses were elicited by sciatic nerve stimulation (1 msec, 0.5/sec), visual evoked responses by light flashes (0.5/sec). A conventional 8-channel EEG apparatus was used to record both the EEG and the chopped DC potentials.

Results. Examples of typical experiments are shown in Figure 1. StSD did not significantly influence the spontaneous EEG, unless it invaded the overlying cortex[6]. As this occurs in only 50% cases and always after a considerable delay (usually 3–5 min, Figure 1A), at least 2–3 min were available for observing the pure striatocortical effects. Visual evoked responses were unaffected by ipsilateral StSD (Figure 1C) during this interval, while somatosensory evoked responses were severely depressed (Figure 1A, B). The decrease of the evoked responses started when the slow potential change in caudate attained maximum. The depression culminated after 10–30 sec, the maximum effect lasted for 20–30 sec and full recovery was reached 30–40 sec later. Both positive and negative components of the evoked response were influenced in the same way. Results of 29 experiments are summarized in Figure 2 showing the average changes of the surface positive components of the somatosensory or visual evoked responses during StSD. The predepression amplitude corresponds to the 100% level and the average curves are synchronized either to the maximum of the slow potential (full line) or to the onset of the maximal depression of the evoked response (dashed line). Synchronization of individual recordings in the latter way made the depression more pronounced. The displacement of the average curve along the time axis corresponded then to the mean delay between the maximum

[1] G. BROŽEK, Physiologia bohemoslov. *15*, 98 (1966).

[2] H. COLLEWIJN and A. VAN HARREVELD, Expl Neurol. *15*, 425 (1966).

[3] A. A. P. LEÃO, J. Neurophysiol. *7*, 359 (1944).

[4] I. SHIMA, E. FIFKOVÁ and J. BUREŠ, J. comp. Neurol. *121*, 485 (1963).

[5] E. FIFKOVÁ and J. MARŠALA, in *Electrophysiological methods in biological research* (Ed. J. BUREŠ, M. PETRÁŇ and J. ZACHAR; Publ. House Czech. Acad. Sci. Prague 1960), p. 426.

[6] E. FIFKOVÁ and J. SYKA, Expl Neurol. *9*, 355 (1964).