equivalent amount of Δ^1-THC administered by itself. The subsequent decline of CBN concentrations was considerably more rapid than those of Δ^1-THC. These facts allow reconciliation of the findings that although CBN has very low activity by injection[1,9-11] it can cause intoxication when infused i.v. at 6 times the dose rate required by Δ^1-THC for intoxication[12]. However a more rapid disappearance of CBN compared to Δ^1-THC also means that equilibrium blood concentrations achieved at any given dose rate will be lower for CBN than Δ^1-THC. It follows that the apparently low activity of CBN is due to its relatively rapid clearance rather than to a low intrinsic activity.

When 0.13 mg CBN was injected with 1 mg Δ^1-THC, the Δ^1-THC blood levels from the mixture were already as low at 3.5 min as they were with Δ^1-THC alone, after 10 min (Figure 1). This suggests that an antagonism for the effects of Δ^1-THC in rats should be observable provided the assumption that there is rapid equilibration of Δ^1-THC between the blood and brain can be made. Such an assumption is not unreasonable since it has been noted that there is not such a blood-brain barrier for Δ^1-THC in mice[13]. Two groups[4,5] have noted an antagonism but detailed comparisons with our work are not possible.

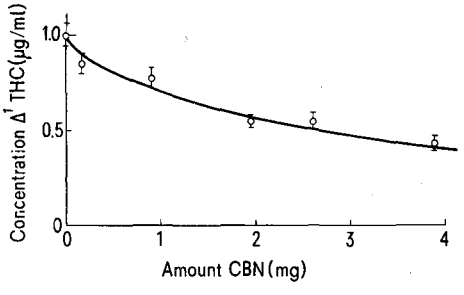

Fig. 3. Δ^1-THC blood levels determined at 3 min after i.v. injection of 1 mg Δ^1-THC and variable amounts of CBN.

Increasing the amounts of CBN introduced with the Δ^1-THC decreased Δ^1-THC blood levels only slightly but increased CBN blood levels in direct proportion. It might be expected therefore, that the CBN in CBN-rich mixtures could contribute significantly to the over-all activity, and relative to mixtures low in CBN but with the same Δ^1-THC, these mixtures could even exhibit an improved activity. This could explain the finding that the amounts of Δ^1-THC in marihuana containing an high proportion of CBN do not fully account for its observed activity[6].

CBN is generally present in 'pure' Δ^1-THC (as an aerial oxidation product), in marihuana, and is an artefact of smoking Δ^1-THC[14]. Our results are therefore of importance for in vivo and behavioural studies.

Summary. The co-administration of cannabinol with Δ^1-tetrahydrocannabinol accelerates the rate of clearance of Δ^1-tetrahydrocannabinol from rat blood. This increased rate of clearance appears to follow that of cannabinol. The implications of these findings are discussed.

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4 March 1975.

[11] L. E. HOLLISTER, Experientia *29*, 825 (1973).
[12] M. PEREZ-REYES, M. C. TIMMONS, K. H. DAVIS and M. E. WALL, Experientia *29*, 1368 (1973).
[13] E. W. GILL and G. JONES, Biochem. Pharmac. *21*, 2237 (1972).
[14] F. MIKES and P. G. WASER, Science *172*, 1158 (1971).
[15] I am most grateful to Professor R. MECHOULAM for helpful discussions during the course of this work. The work was, in part, supported by a grant from the N.I.D.A.
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Effect of Ethyl m-Aminobenzoate (MS-222) on Ampullae of Lorenzini and Lateral-Line Organs

Methane sulphonate of meta-aminobenzoic acid ethylester[1] (MS-222, ethyl-m-aminobenzoate, tricaine methanesulphonate, metacaine methanesulphonate, $C_9H_{11}O_2N + CH_3SO_3H$) is widely used for general anesthesia and sedation of fish and amphibia. (For references see BOVÉ[2]). Belonging to the pharmacological group of local anesthetics, the substance can be expected to act not only on the central but also on the peripheral nervous system. Previous observations (HENSEL and NIER, unpublished) have shown that in cods (*Gadus gadus*) anesthetized with MS-222, the spontaneous activity of lateral-line organs was depressed. The present study was undertaken to investigate more systematically the effect of MS-222 on isolated preparations of ampullae of Lorenzini and lateral-line organs.

Materials and methods. Isolated preparations of the mandibular group of the ampullae of Lorenzini of dogfishes (*Scyliorhinus canicula*) were used. The ampullae were stimulated thermally and electrically as described previously (BROMM, HENSEL and NIER[3], HENSEL[4]). The electric stimuli were applied by means of a microelectrode inserted into the opening of an ampullary canal. Isolated preparations of lateral-line organs were obtained from the mandibular canal of dogfishes (*Scyliorhinus canicula*) and the lateral-line canal of cods (*Gadus gadus*). The preparations were placed on a water-circulated thermode, and afferent single-fibre activity was recorded. Usually the preparations were bathed with saline for selachians or teleosts, respectively, flowing with a constant velocity of ca. 0.02 cm^3 sec^{-1}. MS-222 was added in concentrations of 10^{-3} to $5 \cdot 10^{-5}$ g cm^{-3} to the bathing fluid.

Results. The isolated mandibular ampullae were kept at 19 °C. At this temperature the static discharge has its maximum (HENSEL and NIER[5]). The continuous application of 10^{-3} to $5 \cdot 10^{-5}$ g cm^{-3} MS-222 led to a gradual decrease in the resting discharge of the whole nerve as well as of single fibre preparations, the impulse rate reaching the zero level after 4 to 9 min. No systematic correlation was seen between the concentration applied and the time required for complete inhibition. This might be due to the fact that the size of ampullae, length of ampullary canals, thickness of connective tissue, and location of receptors within the ampullae varied with each preparation. When

[1] Manufacturer: Sandoz AG, Basel.
[2] F. J. Bové, MS-222 SANDOZ (Sandoz AG, Basel).
[3] B. BROMM, H. HENSEL and K. NIER, Pflügers Arch. *347*, R28 (1974).
[4] H. HENSEL, Z. vergl. Physiol. *37*, 509 (1955).
[5] H. HENSEL and K. NIER, Pflügers Arch. *323*, 279 (1971).

MS-222 was replaced by saline, the static discharge started again and usually reached the initial level after 9 to 15 min.

Rapid cooling from 19 °C to 13 °C caused a transient overshoot in the impulse rate of single fibres. During continuous application of MS-222, the static as well as the dynamic responses decreased gradually, the static component being inhibited earlier than the dynamic one. On applying saline, the dynamic as well as the static responses were restored (Figure 1). Correspondingly the static component reappeared later than the dynamic one.

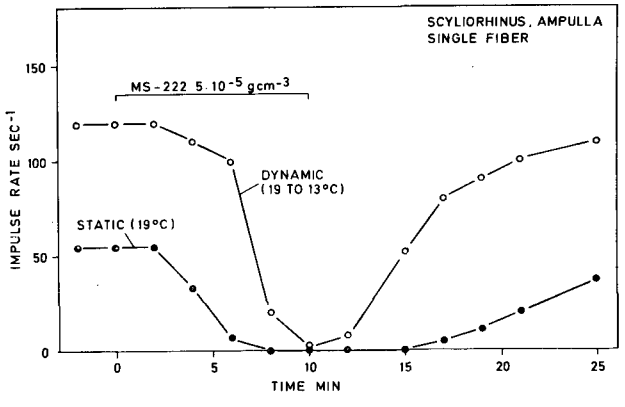

Fig. 1. Static impulse rate at 19 °C and dynamic overshoot on cooling from 19 °C to 13 °C of single fibre from ampulla of Lorenzini in *Scyliorhinus* when applying MS-222. The preparation was bathed with saline before and after MS-222.

Electric d.c. stimuli were applied to the ampullary canal at constant temperature of 19 °C, the inserted microelectrode being positive. In most experiments, the electric stimulus was set 10 times higher than the threshold value. The threshold was defined as the current required for a 10% change of the resting discharge. The onset of the rectangular electric stimulus caused a sudden inhibition of the static discharge, followed by a gradual recovery of activity (Figure 2). This recovery was measured in terms of the mean impulse rate within 3 sec after the onset of the electric stimulus ('on, recovery' in Figure 2). Furthermore, the static impulse rate at 19 °C and the dynamic overshoot ('off-response' in Figure 2) following the offset of the electric stimulus was measured. During application of MS-222 all described parameters decreased, the static component disappearing earlier than the dynamic one.

The isolated lateral line organs of *Gadus* and the mandibular lateral line organs of *Scyliorhinus* were kept at constant temperature of 19 °C. At this temperature they showed a regular static discharge of afferent single fibres (Figure 3). Continuous application of MS-222 led to a reversible inhibition of activity similar to that seen in the ampullae of Lorenzini.

Discussion. The results show that MS-222 in concentrations as low as $5 \cdot 10^{-5}$ g cm^{-3} causes a complete and reversible inhibition of the static discharge, the dynamic overshoot on cooling, and the response to electric stimulation of the ampullae of Lorenzini. The static activity of lateral-line organs is inhibited in a similar way. This action must be accounted for when experiments on sensory nerve endings are carried out in animals anesthetized with MS-222. For example, lateral-line afferents have been recorded in *Xenopus laevis* anesthetized with MS-222 in concentrations of $2 \cdot 10^{-3}$ to $2 \cdot 10^{-4}$ g cm^{-3} (Murray and Capra-

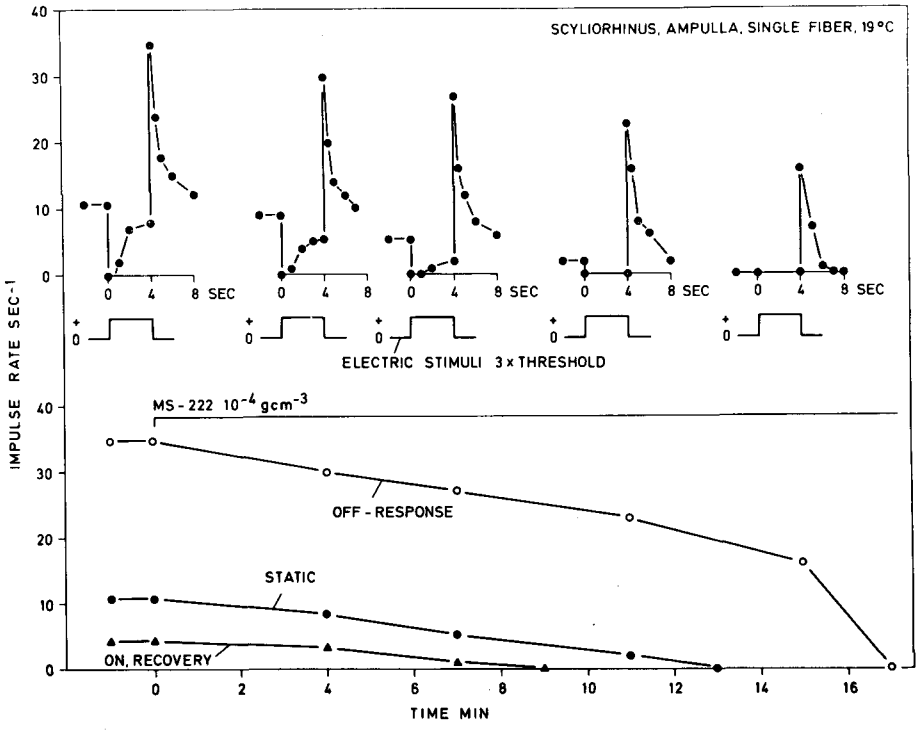

Fig. 2. Response of single fibre from ampulla of Lorenzini in *Scyliorhinus* to electric d.c. stimulation during application of MS-222 at 19 °C. The upper curves show temporal response to electric stimulus at 0, 4, 7, 11 and 15 min. The 3 lower curves show off-response (dynamic overshoot), static discharge at 19 °C, and recovery of impulse rate (on, recovery) during electric stimulation. This parameter is defined by mean impulse rate within 3 sec after onset of stimulus.

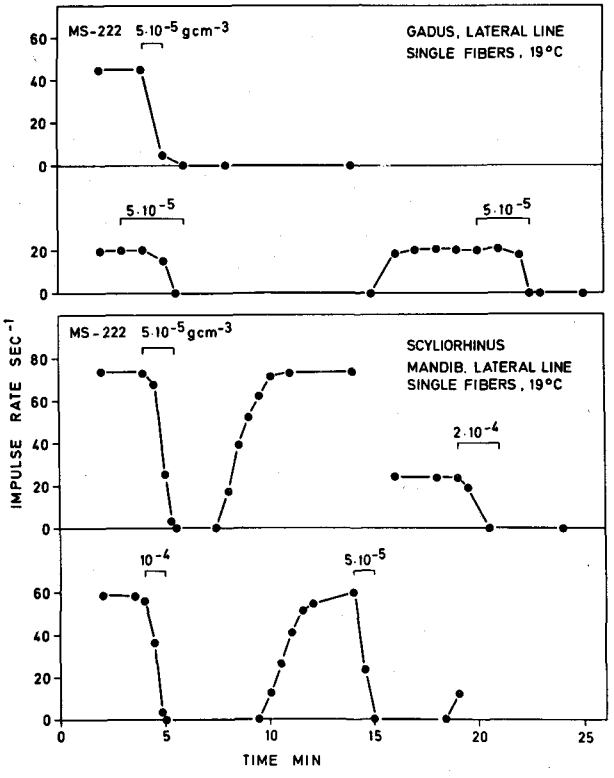

Fig. 3. Static impulse rate at 19 °C of various single fibres from lateral-line organs in *Gadus* and *Scyliorhinus* when applying MS-222. During the remaining time intervals, the preparation was bathed with saline.

NICA[6]). However, in these particular experiments, the disturbance by the anesthetic may possibly be neglected since the isolated lateral-line preparations had been bathed in saline for a longer period of time before impulses were recorded[7,8].

Zusammenfassung. Aethyl-m-Aminobenzoat (MS-222) führt in Konzentrationen von 10^{-3} bis $5 \cdot 10^{-5}$ g cm^{-3} an Lorenzinischen Ampullen zu einer kompletten und reversiblen Hemmung der Spontanaktivität, der dynamischen Reaktion bei Kältesprüngen und der elektrischen Reizantwort. Ebenso hemmt es die Spontanaktivität von Seitenlinienorganen. Diese periphere Wirkungskomponente der als Kaltblüter-Anaestheticum verwendeten Substanz muss bei allen Versuchen an peripheren Rezeptoren berücksichtigt werden.

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[6] M. J. MURRAY and R. R. CAPRANICA, J. comp. Physiol. *87*, 1 (1973).
[7] Supported by the Deutsche Forschungsgemeinschaft.
[8] The experiments were performed at the Biologische Anstalt Helgoland.
[9] Physiologisches Institut der Universität, Martinistr. 52, D-2 Hamburg 20 (Bundesrepublik Deutschland, BRD).

A Central Nervous System Depressant-Antidepressant

Tricyclic antidepressants of the imipramine class are accepted as useful in the treatment of endogenous (involutional) depressive illnesses. Instances of 'pure' involutional depression are in the minority and usually anxiety is associated with depression[1]. Such mixed depressed anxiety states are frequently treated with antidepressants producing marked sedative effects, viz., amitriptyline or doxepin[2] or combinations of an antidepressant and a neuroleptic[3] or anxiolytic[4]. Anxiolytic or even sedative-hypnotic therapy, viz., diazepam or phenobarbital alone is also used[2,5,6].

The present report deals with a new tricyclic agent distinguished by the presence of an allenic side chain. It possesses properties which may be ideally suited for mixed anxiety-depressive therapy. This agent, 5-(3-dimethylaminoprop-1-enylidene)-5H-dibenzo [a, d] cycloheptene maleic acid salt, abbreviated DMPD (IVb), exerts marked antidepressant effects and simultaneously is a potent central nervous system (CNS) depressant.

Methods. Reversal of reserpine (RES) induced hypothermia[7] was used to assess the antidepressant action of DMPD and other agents. Albino mice (18–24 g) were given RES (5 mg/kg, i.p.). 2 h later test material was administered orally. 1 h later and at hourly intervals for 4 h, rectal temperatures were recorded. Percent reversal of hypothermia = (D-R/C-R) 100, where D, R and C refer to mean rectal temperatures of drug treated reserpinized mice, reserpinized untreated mice and control animals, respectively.

DMPD was given i.p. to mice at a dose of 10 mg/kg 30 min after treatment with the MAO inhibitor pargyline (30 mg/kg, p.o.). L-DOPA was given $4^1/_2$ h later to these mice. The degree of CNS excitation evoked in such mice was compared to untreated mice or mice receiving either pargyline and L-DOPA or L-DOPA alone.

Behavioral changes produced in normal mice by compounds under study were also systematically evaluated[8]. Large groups of mice were used to obtain ED_{50} estimates[9,10] of each agent's ability to produce a loss of righting for a 30 sec period.

The effect of DMPD on blood pressure, heart rate and/or nictitating membrane responses was evaluated in 10 pentobarbital-anesthetized cats. In these preparations, alteration of various neurotransmitter and autocoid responses were measured.

[1] L. E. HOLLISTER, Drugs *1*, 189 (1971).
[2] L. E. HOLLISTER, Ann. intern. Med. *79*, 88 (1973).
[3] K. RICKELS, Pharmakopsychiat. Neuro-Psychopharmak. *4*, 308 (1971).
[4] H. E. HARE, JR., J. clin. Pharmacol. *11*, 456 (1971).
[5] B. BLACKWELL, J. Am. med. Ass. *225*, 1637 (1973).
[6] D. WHEATLEY, J. nerv. ment. Dis. *148*, 542 (1969).
[7] B. M. ASKEW, Life Sci. *2*, 725 (1963).
[8] S. IRWIN, Gordon Res. Conf. on Medicinal Chem. (1959), p. 133.
[9] A. P. ROSZKOWSKI, J. Pharmac. *129*, 75 (1960).
[10] J. T. LITCHFIELD, JR. and F. WILCOXON, J. Pharmac. *96*, 99 (1949).