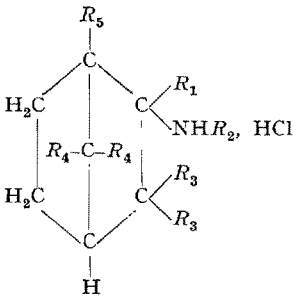
## Hypotensive and Ganglion Blocking Action of Methyl Substituted 3-Amino-norcamphanes

The announcement[1] that 3-methylamino-isocamphane (Mccamylamine) (I) possessed potent ganglion-blocking properties seemed to call for a rather thorough revision of generally accepted concepts of the structure-activity relation of acetylcholine antagonism in autonomic transmission.

A study was undertaken with the object of establishing the structural requirements for appearance of ganglion-blocking action, similar in nature and potency to that exhibited by I, in amines related to this substance.

The results obtained with I and five methyl substituted 3-amino-norcamphanes, congeners of I, represented by the general formula




are shown in the Table.

The results indicate that only minor variations in the number and positions of methyl groups are permissible in order to maintain ganglion-blocking and hypotensive activity. It is evident that the carbon atom at position 2 must be substituted (214/3 is inactive). When this carbon atom is substituted with two methyl groups it is of less importance, but not entirely insignificant, whether there is a C-methyl at position 3 (compare 185/13 [= I] with 214/1). It is also seen that a methyl group at position 4 is permissible (215 is active), but it may also be absent (214/1 is active). The high activity of 185/13 taken together with the paradoxical behaviour of 213 indicate the preference for grouping the substituents in a definite spatial relation to the amine function (not taking *endo-exo* isomerism into account).

The results further indicate a prominent central factor in the hypotensive action of these compounds. Evidence for this is that the blood pressure is still decreased and that the carotis occlusion reflex is absent or suppressed after the nictitating membrane contraction has again reached normal values.

The compounds with pronounced hypotensive action elicit a reversible syndrome of central nervous origin in rats fed the substance for only short periods, while for instance Asa-214/3 does not call forth this syndrome and does not reduce blood pressure. Asa-213 has a quite potent ganglion-blocking action, but has a sustained hypertensive action.

That I is not active by any simple competitive antagonism of acetylcholine has been shown for other pharmacological preparations by BENNETT, TYLER, and ZAIMIS[4].

| Code (Asa) | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | PS[1] ganglion blocking action, % of $C_6$ | S[2] ganglion blocking action | anti Acetylcholine action[3], % of atrop. | BP-action in anesthetized cats[4], dose mm Hg max. depression (each number one experiment) duration | C.O.R.[5] | $LD_{50}$ i.p. mg/kg mice | $LD_{50}$ per os mg/kg mice | Neurotoxic symptoms, rats per os |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 214/3 | H | Me | H | H | H | 10 | (+) | 0·02 | inactive | normal | 352 | | 59 mg/kg/day for 60 days. No abnormal symptoms |
| 214/1 | H | Me | Me | H | H | 300 | +++ | 0·006 | 0·5 mg/kg: – 5, –20, –15, –40, > 2 h<br>1 mg/kg: –20, –20, –10, –65, –30 > 4 h | strongly reduced | 105 | 290 | 52 mg/kg/day for 14 days. Tremor, ataxia, hypermotility |
| 185/13 (= I) | Mc | Me | Me | H | H | 450 | +++ | 0·09 | 1 mg/kg: –60, –30, > 4 h<br>2 mg/kg: –35 > 21/2 | strongly reduced | 39 | 92 | 40 mg/kg/day for 21 days. Tremor and ataxia |
| 214/6 | H | H | Me | H | H | 40 | + | 0·006 | 1 mg/kg: – 4, | reduced | 113 | | |
| 213 | H | Me | H | Me | Me | 130 | + | 0·002 | 3 mg/kg: –18 > 1 h<br>1 mg/kg | 50% | 74 | | |
| 215 | H | Me | Me | H | Me | 135 | ++ | 0·001 | 3 mg/kg: } + 10 to + 30 > 11/2 h<br>5 mg/kg: } > 11/2 h<br>1 mg/kg: –15, > 11/2 h<br>3 mg/kg: –40 (–17 after 51/2 h)<br>5 mg/kg: –20, –30, –40, > 51/2 h | strongly reduced | 51 | 142 | 72 mg/kg/day for 60 days. Tremor, ataxia, hypermotility, convulsions |

[1] Parasympathetic ganglion-blocking action (for details of technique, see [2]). [2] Sympathetic ganglion-blocking action, quantitative expression not possible due to length of action (for details of technique, see [2]). [3] Peripheral anti-acetylcholine action (guinea pig ileum) (technique of MAGNUS[3]). [4] Chloralose-urethane anesthetisized cats, intravenous injection, carotid blood pressure. [5] Carotis-occlusion reflex (cf. [2]).

[1] K. H. BEYER, C. A. STONE, J. E. BAER, and E. J. ZAWOISKI, 20th Int. Physiol. Congress, Bruxelles 1956, Abstracts of Communications, p. 94. – C. A. STONE, M. L. TORCHIANA, A. NAVARRO, and K. H. BEYER, J. Pharmacol. exp. Therap. *117*, 169 (1956).

[2] J. FARSTORP and J. G. A. PEDERSEN, Acta pharm. tox. *10*, 7 (1954). [3] R. MAGNUS, Pflügers Arch. ges. Physiol. *102*, 123 (1904).

These observations taken together with the results presented in this paper support the view that I is not merely a ganglion blocker with a uniform peroral absorption and a prolonged action due to its peculiar recirculation in the organism. (cf. BEYER *et al.*[1]).

Recent evidence[5] incidentally also points to the importance of central factors in the hypotensive action of ganglion-blocking agents representing several of the more 'orthodox' species known to possess such action.

Asa 214/1 was submitted to a brief clinical trial. 7 patients with grave fixed hypertension were treated for 8–33 days. The daily doses were from 15 to 90 mg. Asa 214/1 had a strong hypotensive effect, which, after a single dose, could last more than 12 h. The effect was practically the same after oral and subcutaneous administration. The effective dose was about 3 times as great as by treatment with I.

Parasympathetic side-effects were less pronounced than by treatment with I.

Asa 214/1, however, is unsuited for clinical use, as 5 of the treated patients in the course of 8–16 days displayed symptoms of cerebral disorder: tremor, ataxia and choreiform movements. Moreover, one patient was hallucinated and one developed a grave psychosis. All toxic symptoms disappeared in less than 30 days after discontinuation of the treatment.

Similar cerebral toxic symptoms have been described after treatment with I[6]. The cause of the symptoms is unknown.

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*Zusammenfassung*

5 Methyl-substituierte 3-Amino-norcamphane wurden im Vergleich zu 3-Methylamino-isocamphan in bezug auf Blockierung der Synapsen der autonomen Nervensysteme und blutdrucksenkenden Wirkung im narkotisierten Tier untersucht.

Die Ergebnisse deuten auf eine ganz spezifische Konstitutions- und Konfigurationsabhängigkeit der Wirkung sowie auf einen komplexen pharmakodynamischen Mechanismus, in dem die zentrale Blutdrucksenkung eine nicht unwesentliche Rolle spielt.

Erfahrungen aus kurzzeitigen klinischen Versuchen werden mitgeteilt.

[4] G. BENNETT, C. TYLER, and E. ZAIMIS, Lancet *1957*, II, 218.

[5] H. E. LAPE and J. O. HOPPE, J. Pharmacol. exp. Therap. *116*, 453 (1956). – T. B. O'DELL, C. LUNA, and M. D. NAPOLI, J. Pharmacol. exp. Therap. *114*, 306, 317 (1956). – T. B. O'DELL and M. D. NAPOLI, J. Pharmacol. exp. Therap. *120*, 438 (1957). – A. S. DONTAS and M. NICKERSON, J. Pharmacol. exp. Therap. *120*, 147 (1957).

[6] Q. B. DEMING, M. E. HODES, J. G. EDREIRA, and A. BALTHAZAR, N. Engl. J. Med. *256*, 739 (1957). – H. M. PERRY and H. A. SCHROEDER, J. Amer. Med. Assoc. *164*, 1455 (1957).

## Survival of Bull Spermatozoa after Exposure to 150 atm $N_2$ for 15 Days

In the preservation of living cells through deep freezing, controllable factors are of interest. Among such factors pressure is one that can be easily and rapidly changed. LORANT, LORANT, ANGRIST, and KORPMAN[1], among others, have used high pressure as a means for storing blood at about – 12°C without freezing it. Investigations of high pressures in relation to freezing for the preservation of living cells are not to be found in the literature. MERYMAN[2] has studied blood preservation through deep freezing using blood protected by a moderate amount of dextrose which was sprayed through a nozzle against the surface of liquid nitrogen (– 197°C) and thus rapidly transferred into the frozen state. It has not been possible with MERYMAN's experimental conditions to achieve vitrification as investigated by LUYET[3], but the results when thawing and using the blood for transfusion are good and seem promising even for routine use. Further improvement of the method is thus of interest and could eventually be achieved if spraying was undertaken in an atmosphere under high pressure. Technically this would be possible using hydrogen or helium over liquid nitrogen.

Considering the practical value of vital deep freezing and the need to modify the process in different ways for different materials, it seems interesting to investigate the effect of high gaseous pressure on some living material of relevance in vital deep freezing. One such material is the living sperm cell. Earlier investigators[4] have demonstrated that mammalian tissues, such as skeletal muscle and heart muscle and also blood[5], can tolerate at room temperature hydraulic pressure of at least 1000 atm for short periods and for much longer periods if the temperature is lowered or the pressure not as high. These results are important if high pressure and release of the same is tried as a means of modifying the mechanics of freezing. They make it probable that the pressure itself would not be a first hand limiting factor. For our experiments bull sperm was chosen and was studied under pressure in a gaseous environment (packed in polyethylene tubes). Little or nothing seems to have been published concerning the tolerance of living mammalian cells against gases under high pressures. Our best knowledge seems to have been collected in deep diving and in such research the pressures hardly exeed 15 atm. A good tolerance, against higher gaseous pressures also, is evidently necessary if material for deep freezing is eventually going to be investigated and handled in atmospheres with a very much higher pressure.

As a first step in the investigation of preservative freezing under pressure, the author has tested living bull sperm with respect to the tolerance against two different gases, namely $N_2$ and $H_2$. A maximum pressure of 150 atm was used. Bull sperm was chosen because the preservative freezing of such material is already in routine use in some places and also because it is easily available from the farmers' organisations for artificial insemination. (For

[1] A. LORANT, G. J. LORANT, ANGRIST, and R. KORPMAN, J. clin. Invest. *32*, 1005 (1953).

[2] H. MERYMAN and E. KAFIG, *The Freezing and Thawing of Whole Blood*. Naval. Med. Res. Inst. Rept. Project NM 0000180110 (Bethseda, Md. 1955). – H. T. MERYMAN, Science *124*, 515 (1956).

[3] B. J. LUYET and P. M. GEHENIO, *Life and Death at Low Temperatures* (Biodynamica, Normandy, Mo. 1940).

[4] V. EBBECKE and O. HASENBRING, Pflügers Arch. ges. Physiol. *236*, 405 (1936); *236*, 416 (1936).

[5] R. HAUBRICH, Pflügers Arch. ges. Physiol. *239*, 304 (1938). – A. G. JESSIMAN and C. W. WALTER, Surg. Forum *36*, 529 (1950).