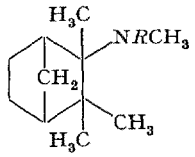
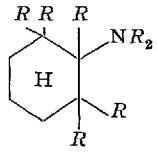
Polymethylcyclohexylamine - neue, hypotensiv wirksame Substanzen[1]

Nach Feststellung der hohen ganglioplegischen und hypotensiven Wirksamkeit von Mecamylamin (I)[2] und Dimecamin (II)[3] hielten wir es für zweckmässig, die Suche nach Substanzen mit ähnlicher Wirksamkeit auf die Gruppe der Polymethylcyclohexylamine auszudehnen, die sich von den Substanzen I und II durch das Fehlen der Methylenbrücke unterscheiden, hingegen mit ihnen die Anhäufung der Methylgruppen in unmittelbarer Nähe des Stickstoffatoms gemeinsam haben. Die Richtigkeit dieses Vorgehens wurde durch kürzlich erschienene Mitteilungen[4,5] über die analoge Wirksamkeit des 1,2,2,6,6-Pentamethylpiperidins (Pempidins) angedeutet, welche die vorläufige Veröffentlichung unserer Ergebnisse beschleunigten.



I, $R = H$
II, $R = CH_3$



III

Die von uns synthetisierten Substanzen können durch die allgemeine Formel III erfasst werden, wobei R Wasserstoffatome oder Methylgruppen darstellen. Sie wurden aus Cyclohexanon, 2-Methyl-, 2,2-Dimethyl-, 2,2,6-Trimethyl- und 2,2,6,6-Tetramethylcyclohexanonen[6,7] dargestellt, und zwar a) durch Reduktion der Oxime mit Natrium und Alkohol (primäre Amine), b) durch Umsetzung mit Methylmagnesiumjodid, Dehydratation, Rittersche Reaktion mit Cyanwasserstoff[8] und nachfolgende Hydrolyse (primäre Amine), c) durch Reduktion der Formamidoderivate mit Lithiumaluminiumhydrid (sekundäre Amine) und d) durch Methylierung der primären Amine mit Formaldehyd und Ameisensäure (tertiäre Amine).

Die von uns dargestellten Substanzen sind in der Tabelle übersichtlich zusammengestellt, wo auch die Schmelzpunkte der Hydrochloride und die vorläufigen Ergebnisse der Bewertung der hypotensiven und ganglioplegischen Wirksamkeit angeführt sind.

Der Einfluss auf den Blutdruck bei der narkotisierten Katze (Chloralose mit Phenobarbital) wird als maximale Druckänderung nach intravenöser Verabreichung von 5 mg/kg der zu untersuchenden Substanz ausgedrückt. Die Blutdruckänderung gegenüber dem Ausgangswert (80–110 mm Hg) um 0–10 mm Hg wird als 0 bewertet, die Senkung um 10–20 mm Hg als –, Anstieg +, um 20–40 mm Hg als –– bzw. ++, über 40 mm Hg als ––– oder +++. Die ganglioplegische Wirkung, die durch die Veränderung der Kontraktionen der präganglionär gereizten Katzennickhaut bewertet wurde, wird bei der Dämpfung der Kontraktionen um 5–25% als –, um 25–50% als ––, um 50–75% als ––– und um 75–100% als ———— ausgedrückt.

Aus der Tabelle geht hervor, dass einige Polymethylcyclohexylamine ähnliche hypotensive und ganglioplegische Wirksamkeit wie Mecamylamin (I) und Dimecamin (II) besitzen. Von ihnen ist besonders das N,N,1,2,2-Pentamethylcyclohexylamin («Penhexamin») gegenwärtig der Gegenstand unserer eingehenderen Untersuchung.

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Hydrochloride der Polymethylcyclohexylamine und ihre Eigenschaften

Substanz Nr.	Cyclohexylamin	Smp. °C	Blutdruckwirkung intravenös (5 mg/kg i. v.)	Ganglioplegische Wirkung intravenös (5 mg/kg i. v.)
III/2862	N,1-Dimethyl	145–146	++	––– a,b
III/2908	*cis*-N,2-Dimethyl	150	0	0
III/2904	*trans*-N,2-Dimethyl	147	0	–
III/2928	2,2-Dimethyl	276	–	––
III/2868	N,N,1-Trimethyl[9]	183	+	————
III/2903	*cis*-N,N,2-Trimethyl	180–181	+	––
III/2895	*trans*-N,N,2-Trimethyl	153–154	±	0[b]
III/2944	N,2,2-Trimethyl	213	––	–
III/2962	1,2,2-Trimethyl	>330	––	————
III/2943	2,2,6-Trimethyl	265	0	0
III/2934	N,N,2,2-Tetramethyl	180–181	––	––
III/2951	N,1,2,2-Tetramethyl	218–219	–	————
III/2917	2,2,6,6-Tetramethyl	>300	–	——— a
III/2961	N,N,1,2,2-Pentamethyl	hygroskopisch	––	————
III/2959	N,N,2,2,6-Pentamethyl	hygroskopisch	–	———
	Mecamylamin-HCl (I-HCl)		––	————
	Dimecamin-HBr (II-HBr)		––	————

[a] Kurzfristige (vorübergehende) Wirkung. [b] Trotz deutlicher Dämpfung der Kontraktionen steigt der Gesamttonus der Nickhaut an.

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Summary

A series of cyclohexylamine derivatives methylated both on nitrogen atom and in position 1, 2 and 6 of the cyclohexane ring have been prepared. Some of them showed considerable hypotensive and ganglioplegic activity. The most interesting compound is N, N, 1, 2, 2-pentamethylcyclohexylamine ('penhexamine') whose activity compares favourably with mecamylamine or dimecamine.

Albumin Synthesis in Regenerating Rat Liver Cells[1]

In a previous report dealing with the effects of the partial hepatectomy on the rat liver soluble proteins (GUIDOTTI and CLERICI[2]), evidence was given that these proteins from the regenerating liver present essentially the same electrophoretic pattern as those from the resting one. However, no reliable data on the albumin behaviour were obtained, because our technique failed to detect this protein.

Since the liver is the site of production of albumin in the rat (MILLER *et al.*[3,4], JENSEN and TARVER[5]), whose albumin concentration in serum is claimed to control liver regeneration (GLINOS and GEY[6], GLINOS[7]), an attempt has now been made to investigate the *in vitro* turnover of the above-mentioned protein in the resting and regenerating rat liver.

Male albino rats, Wistar strain, fed a standard diet, were hepatectomized according to HIGGINS and ANDERSON[8]. After 12 h fasting, normal and operated animals (72 h after hepatectomy) were killed and their livers perfused with saline solution in order to remove completely the blood proteins[2]. Livers were then taken out, sliced, and pooled to obtain 12 g of wet tissue slices. 6 g of tissue slices were added to a ml 100 flask containing 20 ml of a cold Ringer medium[9], and the whole content of the flask was quickly homogenized and lyophilized. The remaining 6 g of tissue slices were added to a second flask containing 20 ml of the above-mentioned medium supplemented with 20 μM of glycine-1-^{14}C (specific activity 1 $\mu c/\mu M$). After 2 h of incubation in air at 38°C, the flask was cooled in a ice bath; the content was then homogenized and lyophilized as indicated above.

Soluble proteins were extracted according to the technique of ADJUTANTIS[10] as modified by CIARANFI and DI SABATO[11]. 0·02 and 0·04 ml of a 7% water solution of proteins from each sample, and 0·02 ml of normal rat serum, were utilized for a paper electrophoresis carried out as described by the above mentioned authors[10,11]. All the steps of this procedure were performed in a cold room (+ 2°C).

Amidoschwarz-stained strips were photometrically scanned by means of an Elphor photometer, and the percentage of the protein content was evaluated by planimetry. The albumin-carrying section of each strip was separated from the other soluble proteins and hydrolized with HCl 5 *N* in sealed ampulles kept at 116°C for 16 h. The cloudy suspension obtained thereafter was centrifuged; the supernatant fluid, poured into suitable polythene planchets, was dried under a current of warm air. Radioactivity measurements were performed by means of a windowless flow counter; counts were corrected for self absorption.

After incubation, a protein peak, whose migration rate was similar to that of the serum albumin, was always detected in both the electrophoretic patterns from resting and regenerating rat liver slices. Therefore, this peak, constantly lacking in the electrophoretic patterns from non-incubated liver slices[2], was tentatively identified with albumin, newly released.

As for the evaluation of the amount of albumin released during incubation, some errors may arise from the following causes: (1) protein loss during the extraction procedure; (2) inaccuracy of photometric readings due to different affinity of the several proteins towards Amidoschwarz stain; (3) uncertain separation of the overlapping planimetric peaks, and (4) contamination of the albumin peak from α_1-globulin. It has been estimated, however by experience, that with reasonable care these errors do not exceed ± 10–15% of the found values. Radioactivity measurements, which were referred to a mg of albumin, are more reliable because not biased by the main error source, i.e. by number (1).

The results concerning the amount of albumin released during incubation and the incorporation rate of the labelled glycine into the same protein are presented in the Table.

It appears that, during incubation, less albumin is released from the regenerating rat liver, as compared to the resting one. However, the difference is not statistically significant ($P > 0{\cdot}05$).

Furthermore, the glycine incorporation rate into the albumin released from the regenerating liver significantly increases over the resting one ($P < 0{\cdot}01$).

[1] This investigation was aided by a grant from C.N.R., Roma, and was made during a tenure by one of us (G.G.) of a Scholarship from C.N.R., Roma.

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Table

Type of tissue used	Number of experiments	Albumin release (mg/g of wet tissue/h)	Glycine-1-^{14}C incorporation into albumin (counts/min/mg)
Resting liver	7	0·23 ± 0·04*	23·8 ± 3·0
Regenerating liver	6	0·18 ± 0·01	55·3 ± 4·6

* Mean ± S.E.