Comparative Cardio-Inhibitory Effects of Substituted Cyclohexanols

By HAROLD H. SMOOKLER and JOHN J. DEFEO

Cyclohexanol and its derivatives 2-(*o*-tolyl), 2-(*m*-tolyl), 2-(*p*-tolyl), 2-(*o*-chlorophenyl), 2-(*m*-chlorophenyl), and 2-(*p*-chlorophenyl) were tested for cardioplegic effects on intact anesthetized rats, isolated guinea pig hearts, and isolated rabbit atria. Most of the compounds produced inhibitory effects varying from a slight depression to complete arrest. Evaluation of potency was based on the ability to produce arrest and the duration of arrest. In both series of substitutions it appears that the *trans*-2-*o*-derivatives are most potent with the *cis*-2-*o*-derivatives least potent. The 2-*m*- and 2-(*p*-chlorophenyl) compounds appear to be toxic to the myocardium as the hearts do not return to their normal level of activity. Mechanism studies indicate an antagonistic effect between these compounds and calcium.

CARDIOPLEGIC AGENTS have become of interest in the past several years with the advances made in "open heart" surgery techniques employing extracorporeal circulation. Such agents would be of great help in securing a motionless field for surgical repair of various complicated cardiac defects.

Elective cardiac arrest has been attempted with such substances as potassium citrate and acetylcholine (1–4). Opinions on the use of cardioplegic agents vary from skepticism to acceptance for routine use.

Although cyclohexanol has never been reported as having cardioplegic properties, it is evident from various reports that certain derivatives bring about such an effect.

In 1957 (5) it was reported that 2-(*o*-methyl phenyl)-cyclohexanol [actually 2-(*o*-tolyl) cyclohexanol] had been able to arrest the heart of a dog but recovery was unsuccessful.

Huitric (6) and his co-workers published a note concerning the production of mechanical cardiac arrest with 2-(*o*-tolyl)-cyclohexanol. During the course of his work, Huitric had synthesized a series of substituted cyclohexanols (Fig. 1) which were used for this study of the comparative effects of the various substitutions on their ability to produce cardiac arrest.

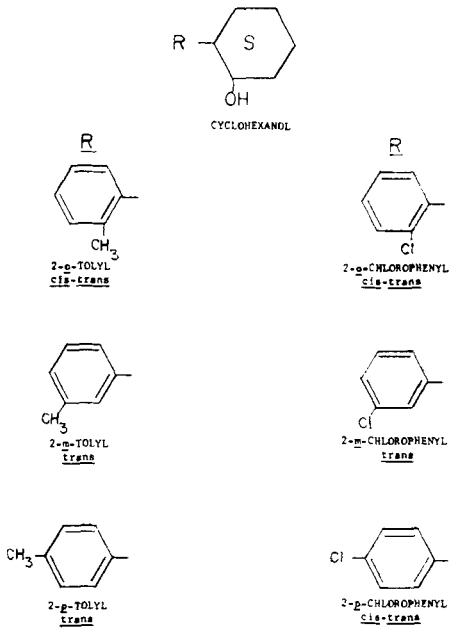

Fig. 1.—Cyclohexanol and its derivatives.

METHODS

The compounds whose structures appear in Fig. 1 have a very low solubility in water. In the tolyl series the *trans*-2-(*p*-tolyl) compound was the only solid, while others were liquid. In the chlorophenyl series, all were solid except the *cis*-2-(*o*-chlorophenyl) compound which was a liquid.

The solid materials were dissolved in 10–50% propylene glycol-water mixtures. An aliquot was measured and diluted to a standard concentration and volume. The liquid forms were mixed with water in order to obtain saturated solutions. A portion of the saturated solution was filtered and the filtrate was employed for testing.

The solubilities of the liquid substances were determined and are listed in Table I. Cyclohexanol was dissolved in water in varying concentrations.

Blood Pressure Studies.—The experiments were conducted in albino rats of both sexes. They were anesthetized with an intraperitoneal injection of 1.2 Gm./Kg. of urethan. The effects were recorded by direct cannulation of the carotid artery through a mercury manometer connected by plastic tubing filled with 7.5% sodium citrate solution. The compounds were administered intravenously. These studies were employed only to determine the rapidity

Received April 28, 1961, from the Department of Pharmacology, College of Pharmacy, University of Rhode Island, Kingston.
Accepted for publication October 20, 1961.
Supported in part by a grant from the Rhode Island Heart Association.
Abstracted from a thesis submitted by H. H. Smookler in partial fulfillment of the requirements for the M.S. degree, 1961.
Presented to the Scientific Section, A.PH.A., Chicago meeting, April 1961.

TABLE I.—WATER SOLUBILITY OF CYCLOHEXANOL AND ITS LIQUID 2-ARYL SUBSTITUTION PRODUCTS

Compound	Physical State	Water Solubility, mg./ml.
Cyclohexanol	liquid	5.67 at 15°[a]
trans-2-(*o*-Tolyl)cyclohexanol	liquid	0.8[b]
cis-2-(*o*-Tolyl)cyclohexanol	liquid	0.5
trans-2-(*m*-Tolyl)cyclohexanol	liquid	1.0
cis-2-(*o*-Chlorophenyl)cyclohexanol	liquid	0.2

[a] Handbook of Chemistry and Physics, 35th ed. (1953–1954). [b] Value reported by Huitric, *et al.* (6).

of effect in the intact animal. Those compounds dissolved in the propylene glycol could not be tested, as the solvent has a pronounced hypotensive effect and thus would mask the effect of the compounds.

Guinea Pig Heart Perfusion.—Guinea pigs of either sex were sacrified with a blow to the head. The heart was removed rapidly and flushed with the perfusing solution. It was then suspended in an Anderson-Craver perfusion apparatus by tying a glass cannula into the aorta. Chenoweth's solution (7) modified by replacing magnesium chloride with an equivalent amount of magnesium sulfate at 37.5°, and saturated with a steady flow of a 95% oxygen, 5% carbon dioxide mixture was employed as the perfusing fluid. The total volume of fluid containing the test compound injected into the heart was kept constant at 2 ml. This placed a limit on the upper level of dosage for the liquid compounds because of their poor solubility. An equivalent mixture of propylene glycol and water was used as a control prior to each injection of the compounds dissolved in this material in order to determine its effects.

The magnitude of effect, the duration of the effect, and the time for recovery to 50% of the normal amplitude was determined following each injection of the test compounds.

Isolated Rabbit Atria.—Rabbits were sacrificed and their hearts removed rapidly. The atria were resected intact and suspended in a (70 ml.) tissue bath containing the same nutrient solution as used in the perfusion experiments. The temperature was maintained at 32° in order to reduce the rate and to increase the amplitude of contractions (8).

A 4.0% solution of calcium chloride was used to determine possible interactions with *trans*-2-(*o*-tolyl)-cyclohexanol, which was selected because it appeared to be the most effective of the compounds tested. All drugs were allowed to act for exactly 3-minute periods before adding other agents or washing.

trans-2-(*o*-Tolyl)-cyclohexanol produced an effect lasting indefinitely when not washed. Calcium chloride produced an effect that lasted for at least 30 minutes if not washed.

The experiments were conducted so that the effects of the experimental compounds were recorded well within the above periods in order to assure ourselves that the effects were due to the drugs alone.

RESULTS

Cyclohexanol in doses of 5 mg./Kg. to 100 mg./Kg. produced a drop in mean blood pressure ranging from 20 to 90% of the normal.

trans-2-(*o*-Tolyl)-cyclohexanol in doses of 0.5 to 4.0 mg./Kg. produced a drop in mean blood pressure of 38 to 83%.

cis-2-(*o*-Tolyl)-cyclohexanol in doses of 1.0 to 3.0 mg./Kg. produced a drop in mean blood pressure of 27 to 74%.

trans-2-(*m*-Tolyl)-cyclohexanol in doses of 1.0 to 6.0 mg./Kg. produced a drop in mean blood pressure of 18 to 99%.

cis-2-(*o*-Chlorophenyl) in doses of 0.5 to 1.5 mg./Kg. produced a drop in mean blood pressure of 37 to 91%.

In all of the above experiments death occurred at the highest dose when this quantity was readministered in the same animal. At the conclusion of all of the tests, urine was aspirated from the bladder and hematuria was found in every case.

The results obtained in the guinea pig heart perfusion studies are shown in Table II. It should be noted that a normal heart will contract from 2 to 3 hours in the apparatus before signs of failure appear. In all of the experiments the compounds acted within 1 minute.

Recovery was allowed to occur spontaneously in order to compare the rates of recovery from the effects of the various compounds. Recovery to a 50% level of the original amplitude was variable, ranging from 3 to 29 seconds for cyclohexanol to 34 to 430 seconds for *trans*-2-(*o*-tolyl)-cyclohexanol.

TABLE II.—COMPARATIVE EFFECTS OF CYCLOHEXANOL AND ITS DERIVATIVES ON THE ISOLATED GUINEA PIG HEART

Compound	Minimum[a] Effective Dose, mg.	Cardiac Arrest Arrests/ Trials	Duration of Arrest Mean and Range, sec.	Mean % Depression ± S.D. (if no arrest)
Cyclohexanol	8.0	7/7	18(9–58)	...
cis-2-*o*-Tolylcyclohexanol	0.5[b]	0/25	...	63 ± 17
trans-2-*o*-Tolylcyclohexanol	0.4	5/6	12(7–14)	85
trans-2-*m*-Tolylcyclohexanol	1.0	5/18	33(11–53)	85 ± 7
trans-2-*p*-Tolylcyclohexanol	1.5	7/16	170(37–275)	88 ± 6
cis-2-*o*-Chlorophenylcyclohexanol	0.2[b]	0/12	...	26 ± 12–93(4)[c]
trans-2-*o*-Chlorophenylcyclohexanol	1.0	9/11	88(29–167)	93 ± 1
trans-2-*m*-Chlorophenylcyclohexanol	1.0	3/8	197(62–284)	62 ± 16
cis-2-*p*-Chlorophenylcyclohexanol	2.0	3/6	262(148–324)	71 ± 18–100(1)[d]
trans-2-*p*-Chlorophenylcyclohexanol	2.0[e]	4/4	442(182–645)	...

[a] Minimum dose which will produce cardiac arrest. [b] Maximum allowable dose injected but not the minimum effective dose. [c] Stimulation occurred in 4 of 12 doses with a mean of 93%. [d] Stimulation occurred in 1 of 6 doses. [e] Toxic dose.

There is an indication of toxicity produced by the *trans*-2-(chlorophenyl) compounds since many of the hearts did not return to normal following the arrest periods.

cis-2-(*o*-Chlorophenyl)-cyclohexanol acted in a manner differing from the other compounds in that it produced a stimulating effect up to 93% of the normal in some hearts. In other cases it was only slightly depressant.

trans-2-(*o*-Tolyl)-cyclohexanol was selected for preliminary mechanism studies due to its reproducible action. A dose of 0.6 mg. was found to be immediately effective in reducing the amplitude of the atrial contractions by 50% without apparently affecting the rate.

It was noted that 4 mg. of calcium chloride in the 70-ml. bath was sufficient to increase the amplitude of the atrial contractions by 100%. When *trans*-2-(*o*-tolyl)-cyclohexanol (0.6 mg.) had reduced the amplitude of contractions by 50% it was found that it required three successive doses of 8 mg. each of calcium chloride over 9 minutes in order to restore the amplitude of contraction, stepwise, to a normal level. When a total of the above amounts (24 mg.) was administered at one time there was a continuous recovery within 3 minutes.

In other tests, when *trans*-2-(*o*-tolyl)-cyclohexanol (0.6 mg.) followed calcium chloride (4 mg.) it was observed that the onset of action was delayed several minutes and the inhibitory effect which followed only overcame the effect of the calcium, thus restoring the amplitude of contractions to a normal or slightly below normal level.

DISCUSSION

The *trans* isomers of these compounds are those in which both the substituted groups and the hydroxyl group occupy equatorial positions on the cyclohexane ring. The *cis* isomers are those where the substituted groups are equatorial and the hydroxyl group is axial to the cyclohexane ring (9).

It is evident that cyclohexanol and its liquid 2-aryl derivatives can produce an immediate drop in blood pressure. The magnitude of the effect is somewhat proportional to the dose. The substitution of a tolyl or chlorophenyl moiety in the number 2 position of the cyclohexanol ring increases the hypotensive activity from 6 to 40 times. This is no doubt due to the inhibitory effect of these substitutions on the myocardium as was shown in the isolated heart tests.

At the dose levels employed in these tests it appears that substitutions at the 2 position on the cyclohexanol structure increase the cardio-inhibitory effect from 4 to 20 times (see Table II). The *trans*-2-(*o*-tolyl) substitution is apparently the most effective in producing arrest at the lowest dose followed by a good recovery. The *cis* isomers of both the (*o*-tolyl) and (*o*-chlorophenyl) compounds are the least depressant, while the *trans* isomers of these compounds are the most effective in their respective series. In both series it appears that the inhibitory effect is enhanced by shifting the methyl on the tolyl and the chlorine on the chlorophenyl from *para* to *meta* to *ortho* positions, respectively.

Tentative conclusions indicate that the tolyl derivatives are less toxic than the chlorophenyl derivatives as evidenced by the failure of most hearts to recover from the effects of the latter compounds.

These facts indicate the importance of the spatial configuration of these compounds in producing their cardio-inhibitory actions.

The antagonism between the calcium ion and *trans*-2-(*o*-tolyl)-cyclohexanol is apparently of a physiological type. The 2-(*o*-tolyl)-cyclohexanol action appears to be the most potent as the same amount of this compound was able to counteract the effect of various amounts of calcium. However, when enough calcium was added to the bath following the depressant effect, it was able to restore the contractions of the atria to a normal level. The major effect of calcium was to increase the latent period of 2-(*o*-tolyl)-cyclohexanol.

SUMMARY

1. Tolyl and chlorophenyl substitutions at the 2 position of the cyclohexanol structure enhance the cardio-inhibitory effect of this compound.

2. The *cis* isomers of the (*o*-tolyl) and (*o*-chlorophenyl) compounds are least effective, whereas the *trans* isomers of these compounds are the most effective in producing cardio-inhibitory effects. The inhibitory effect increases with the position of the methyl and chlorine from *para* to *meta* to *ortho*.

3. The chlorophenyl substituted derivatives appear to exhibit a greater cardio-toxicity than the tolyl derivatives.

4. Calcium delays the onset of action of 2-(*o*-tolyl)-cyclohexanol and antagonizes its effect when administered after the depressant action of the compound has occurred.

REFERENCES

(1) Effler, D. B., Groves, L. K., Sones, F. M., and Kolf, W. J., *Cleveland Clinic Quart.*, **23**, 105(1956).
(2) Lam, C. R., Gahagan, T., Sergant, C. K., and Green, E., *Ann. Surg.*, **146**, 439(1957).
(3) Effler, D. F., *et al.*, *J. Thoracic Surg.*, **34**, 500(1957).
(4) Helmsworth, J. A., Shabetai, R. W., and Margalian, J., *ibid.*, **36**, 214(1958).
(5) Nelson, R., "Extracorporeal Circulation," Charles C Thomas, Springfield, Ill., 1958, p. 487.
(6) Huitric, A. C., West, T. C., Durbin, R. A., and Bryan, G. H., THIS JOURNAL, **48**, 131(1959).
(7) Chenoweth, M. B., and Koelle, E. S., *J. Lab. Clin. Med.*, **31**, 600(1946).
(8) Webb, J. L., *Brit. J. Pharmacol.*, **5**, 87(1950).
(9) Huitric, A. C., personal communication.