A STUDY ON THE STRUCTURE-ACTIVITY RELATIONSHIP OF THE CARDIOTONIC STEROIDS*

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According to the prevailing opinion of today about the structure-activity relationship of cardenolides and bufadienolides, the structural requirements for the characteristic cardiotonic action of these compounds can be summarized as follows: cis-fusion of the C and D rings, hydroxyl groups in positions 3β and 14β , and a five- or six-membered unsaturated lactone ring having β -configuration at C_{14} (1). Most of the experimental data referred to as evidences supporting this opinion have come, however, from the determination of toxic doses in the whole animal, mainly in the cat, rather than from observations of the action of these compounds in isolated hearts. It follows as a natural consequence that the compounds with a rather weak toxicity have been condemned to be inactive, even if they have a strong cardiotonic action, in spite of the fact that such a kind of compounds may actually be more important from the clinical point of view. For example, the compound like dihydrodigitoxin of which the lactone ring is saturated, and the compound like resibufogenin, which have an epoxide ring between C_{14} and C_{15} instead of C_{14} -OH, generally believed to be inactive based on the toxicity data in the cat, have recently been found to be fairly active when tested in the isolated heart (2-3). The authors also found that, different from the above-cited opinion, the presence of 3β -OH is not indispensable for the cardiotonic action of the cardenolides; 3-epi-digitoxigenin was found to have a definite cardiotonic action upon the isolated frog's heart (See Fig. 8). These findings seem to warrant reexamination of the problem, on the basis of the action of these compounds in the isolated heart.

In recent years several adrenocortical steroids and aldosterone antagonists have been reported to be cardiotonic (4-6), despite the fact that their stereochemical structures are essentially different from those of the cardiotonic steroids. In view of these, the authors took up the problem of the cis-fusion of the C and D rings out of the several

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* By the word "cardiotonic steroids" are meant in this paper the steroids of the cardenolides and bufadienolides type and their glycosides.

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important points in the structure of cardiotonic steroids as discussed above and re-examined its importance for the specific cardiotonic action using the isolated frog's heart and the heart-lung preparation of the dog.

METHODS

Experiments were performed on the heart-lung preparation (HLP) of the dog, the details of which were described before (7) and on the isolated frog's heart (Straub's preparation).

Impairment of the heart contractile force was induced by administering an appropriate amount of pentobarbital sodium in the dog experiments, and by reducing the calcium concentration of the bathing medium to one third (0.6 mM) of the normal in the frog experiments.

Compounds used were: DOC-glucoside (Schering A.G.) (DOC)*, aldosterone*, SC-9420 (spironolactone)**, 14-deoxy-14 α -digitoxigenin, (14 α -H D-genin)***, 14 β , 15 β -epoxy-(β)-anhydrodigitoxigenin (14, 15 β -epoxy D-genin)****, 14 α , 15 α -epoxy-(β)-anhydrodigitoxigenin (14, 15 α -epoxy D-genin)****, adynerin*****, 15 α -hydroxydigitoxigenin (15 α -OH D-genin)*****. The last compound was synthesized and supplied by Dr. M. Okada of

FIG. 1. The chemical structure of the compounds used.

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***** A preliminary report on the cardiac action of this compound appeared in *Experientia* **20**, 534 (1964).

FIG. 2. The stereochemistry of the compounds used.

Tokyo Biochemical Institute, Tokyo. For the detailed informations of this compound refer to (8, 9). Chemical structure of all these compounds are shown in Figs. 1 and 2. Stock solutions (10^{-3} g/ml in ethanol) of these compounds were diluted with physiological saline to an appropriate volume in dog experiments and with Ringer's solution to desired concentrations in frog experiments.

The compounds which were found ineffective even in a cumulative dose of 1 mg in the HLP, and at a concentration of 10^{-8} in the frog's heart were judged to be inactive.

At least three experiments were performed with every compound tested both in the HLP of the dog and in the isolated frog's heart.

RESULTS

1. HLP of the dog

1) Cardiac action of the adrenocortical steroids

As is shown in Fig. 3, such representative mineral corticoids as aldosterone and DOC were without any remarkable effect on the inotropic and chronotropic properties of the heart. An aldosterone antagonist, spironolactone, also failed to induce an improvement of heart contractility even in a dose of 1 mg.

FIG. 3. The effect of DOC and aldosterone upon the heart-lung preparation of the dog.

The tracings are from top to bottom : arterial pressure (AP), right atrial pressure (RAP) and time in 1 minute interval.

PB=pentobarbital sodium, DOC=DOC-glucoside, HR=heart rate per minute, SOP=systemic output in ml per minute.

FIG. 4. The cardiac action of 14, 15 β -epoxy digitoxigenin.

14, 15 β -epoxy D-genin = 14 β , 15 β -epoxy-(β)-anhydrodigitoxigenin,
D-genin=digitoxigenin.

Other abbreviations used are the same as in Fig. 3.

FIG. 5. Lack of the cardiotoxic action in 14,15- α -epoxy digitoxigenin.
 14,15- α -epoxy D-genin = 14,15- α -epoxy-(β)-anhydrodigitoxigenin.
 Note the absence of fall in the right atrial pressure!

FIG. 6. The action of 15- α -hydroxydigitoxigenin on the heart-lung preparation.
 15- α -OH D-genin = 15- β -hydroxydigitoxigenin.
 The tracing at the bottom in this figure represents systemic output recorded with a Weese stromuhr.

2) The effect of several cardiotoxic steroids

Injection of 14,15-epoxy D-genin to the rubber tubing leading to the venous cannula resulted in a definite decrease in the right atrial pressure, although the duration of this

effect was rather short (Fig. 4). In contrast, the administration of 14, 15 α -epoxy isomer, even in a cumulative dose of as high as 1 mg, did not produce any remarkable effect on the heart contractility, in contradiction to the finding of Hofer *et al.* (10); there was no decrease in the right atrial pressure (Fig. 5). Slight increase in the right atrial pressure seen in this figure is due to ethanol used as a solvent.

14 α -H D-genin, which has also the trans-fusion at C and D junction like 14, 15 α -epoxy D-genin just mentioned, was also completely devoid of any cardiotoxic activity in the HLP of the dog, further confirming the importance of cis-fusion at C and D junction.

These data add to the importance of the cis-fusion at C and D junction. Nevertheless it seems that the presence of cis-fusion alone is not enough, if the typical cardiotoxic action is to be retained; 15 α -OH D-genin, which has all the characteristic features of digitoxigenin, except that it has another hydroxyl group at 15 α -position, could not produce any improvement of the heart contractile force (Fig. 6). The lack of cardiotoxic action in adynerin, as is shown in Fig. 7 came as another evidence, since this compound still retains cis-fusion at C and D junction, although it has an epoxide ring between C₈ and C₁₄ (11).

II. Isolated frog's heart

Fig. 8 summarizes the effects of all the above-mentioned compounds on the isolated frog's heart. As can be seen from this figure, all the cardiotoxic steroids that were found to be cardiotoxic in the HLP of the dog, were also effective in improving the contractility of the frog's heart, while those compounds which were judged to lack cardiotoxic activity in HLP, were also ineffective in the isolated frog's heart.

FIG. 7. Cardiac action of adynerin.

The tracing marked as SOP represents systemic output recorded with a Weese stromuhr.

FIG. 8. The action of the several cardenolides upon the isolated frog's heart (Straub's preparation).

The concentrations of the compounds used were : D-genin and 14,15 β -epoxy D-genin = 3×10^{-6} ; 14,15 α -epoxy D-genin, 3-epi D-genin, 15 α -OH D-genin, 14 α -H D-genin and adynerin = 10^{-5} .

To induce the impairment of the heart contractility, the concentration of calcium in the bathing medium was reduced to 1/3 (0.6 mM).

Abbreviations used in this figure are the same as in the previous figures.

DISCUSSION

The present data clearly demonstrated that those compounds which have trans-configuration at the C and D junction, including adreno-cortical steroids, did not produce any remarkable cardiotonic activity such as can be elicited by cardiotonic

steroids. Thus, it may be concluded that the *cis*-fusion of the C and D rings, which makes the cardiotonic steroid unique in its stereochemical structure among the many naturally-occurring steroids, is indispensable for its unique cardiotonic activity. The idea that the presence of 14β -OH is essential for the cardiotonic action seems to have been too rigorous, since 14,15 β -epoxy compounds, like 14,15 β -epoxy D-genin and resibufogenin, elicited a clear-cut cardiotonic action, in spite of the fact that they have no OH radical at C₁₄. According to Ragab *et al.* (12), 14 β -artebufogenin, which has H instead of OH at C₁₄, is fairly toxic in the cat, while its isomer, 14 α -artebufogenin is innocuous in the same animal. K.K. Chen, who took a rather serious view of the importance of 14 β -OH (13), later changed his opinion and stated that the *cis*-configuration at C and D junction is more important for the cardiotonic action than the presence or absence of 14 β -OH (14, 15). The same opinion was independently stated by Okada *et al.* (3).

The presence of the *cis*-configuration at C/D, however, is not enough for the preservation of the cardiotonic action. Introduction of α -OH to C₁₅ to make 15 α -OH D-genin completely deprived the parent compound of its cardiotonic action. The formation of the epoxide ring between C₈ and C₁₄ also destroyed the cardiotonic action of digitoxigenin, in spite of the fact that the *cis*-configuration at C/D junction is still maintained. The precise mechanism of this effect is unknown. Presumably, not only the *cis*-configuration at C/D junction, but some special steric arrangements in the vicinity of C₁₄ position seems to be indispensable, if the characteristic cardiotonic action is to be retained.

There is no doubt that the systematic study of K.K. Chen and his coworkers and the quantitative data they have accumulated have played an extremely important role in the recent progress in the chemistry and pharmacology of cardiotonic steroids. The findings stated in this paper indicate, however, that many problems about the structure-activity relationship still await reexamination, on the basis of the action of these compounds on isolated hearts.

In recent years increasing number of papers have been published concerning the cardiac action of the adreno-cortical steroids. In some of these (4-6) it was concluded that the adreno-cortical steroids, especially the so-called mineral corticoids could produce the cardiotonic action similar to that of the cardiotonic steroids. The present authors could not find such a cardiotonic action in the HLP of the dog. This is not at all surprising, since, apart from the absence of the lactone ring, which some people believe to be the most important portion in the molecule of cardiotonic steroids (16), the stereochemistry of the A/B and C/D ring junctions of the adrenocortical steroids is quite different from those of the cardiotonic steroids; in the latter, both these junctions are *cis*, while in the former they are both *trans*. Thus, despite the apparent resemblance of the structural formulae as usually written, in three-dimensional structure, the cardiotonic steroids are a bunched up molecule with both the A- and D-rings strongly "bent" back towards the α -side of the plane of the B- and C-rings, whereas the adreno-

cortical steroids are more or less planar in structure (Fig. 2). In view of this it is quite unlikely, although not impossible, that these two types of compounds possess the similar cardiac action in common.

The reason why some investigators found the positive inotropic action in the adrenocortical steroids is not necessarily clear. The main difference in the experimental methods of the present authors and those investigators consisted in the use of the blood as a perfusion medium by the former and artificial salt solution by the latter. The loss of the substance soluble both in alcohol and ether from the heart perfused with artificial salt solution was demonstrated as early as 1913-1914 by Clark (17). Tanz (18) observed a rather drastic histological damage in the heart forced to contract in an artificial salt solution for a fairly long time, which was successfully prevented by adding certain adrenocortical steroids. These steroids might have substituted the lost lipids in such a preparation and might have maintained the histological integrity of the tissues. The heart muscle preparation, on which adrenocortical steroids were effective, might have been in a special type of failure as a result of the breakdown of this integrity, which could be successfully corrected with adreno-cortical steroids. Cardiotonic steroids are also effective in improving this type of heart failure. But there is another heart failure, on which only the cardiotonic steroids are effective and which is far more important from the clinical point of view.

SUMMARY

To reexamine the importance of cis-fusion of C and D rings for the cardiotonic action of cardenolides and bufadienolides, the cardiac actions of several key compounds of the cardenolides type and representative adrenocortical steroids were studied using the dog heart-lung preparation and the isolated frog's heart. It was concluded that the cis-fusion at this junction is indispensable for the cardiotonic action of these compounds. However, two compounds were found which lacked cardiotonic activity, despite the fact they have cis-configuration at C and D junction, suggesting the importance of a special steric arrangement in the vicinity of C₁, in addition to the cis-configuration of the C/D junction.

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