Br. J. Pharmac. (1969), 37, 485–488.

Effect of spironolactone and norbolethone on the toxicity of digitalis compounds in the rat

H. SELYE, I. MÉCS AND T. TAMURA

Institute of Experimental Medicine and Surgery, University of Montreal, Montreal, Canada

1. In the rat, both spironolactone (an antimineralocorticoid) and norbolethone (an anabolic steroid) inhibit the characteristic neuromuscular disturbances and the mortality produced by digitoxin, gitalin, proscillaridin, digoxin and digitalin. The corresponding effects of strophanthin K, ouabain and digitoxigenin could not be prevented.

2. It may be concluded that, in our experimental conditions, both spironolactone and norbolethone counteract the toxicity of some, but not all, digitalis compounds tested and that both the protective steroids affect the toxicity of the same digitalis derivatives.

3. This antidigitalis effect is not merely a secondary consequence of either antimineralocorticoid or anabolic potency because spironolactone lacks anabolic and norbolethone lacks antimineralocorticoid properties. Indeed, it appears that the ability to antagonize the toxicity of digitalis compounds represents a pharmacological property independent of all known steroid hormone actions, since spironolactone is virtually devoid of these.

It has been noted recently that digitoxin poisoning can be prevented in the rat by pretreatement with certain steroids exhibiting either antimineralocorticoid (for example, spironolactone) or anabolic (for example, norbolethone) properties (Selye, Jelinek & Krajny, 1969). Since the antimineralocorticoids possess no anabolic effects and the anabolic steroids no antimineralocorticoid activity, however, the newly discovered antidigitoxin action is obviously not inseparably linked to either of these two hormone-related properties. Indeed, spironolactone (one of the most active antidigitoxin compounds), though a potent antagonist of mineralocorticoids, is virtually devoid of all classical hormone actions (Kagawa, 1964). Hence, the ability to counteract digitoxin is apparently independent of the known steroid hormone actions. The experiments to be reported here were performed to determine whether spironolactone and norbolethone would also protect against the toxic effects of other digitalis compounds.

Methods

Three hundred and sixty female Sprague-Dawley rats with an initial body weight of 100 g (90–110 g) were divided into thirty-six groups and treated with the various digitalis preparations listed in Tables 1 and 2. As indicators of toxicity we

registered the neuromotor disturbances characteristic of severe digitalis intoxication and the mortality rates. First,preliminary experiments were performed to determine the optimum dosage, route of administration, and the time at which the neuromotor disturbances were most obvious (" time of reading "); since these differed with the various digitalis compounds employed, they are likewise indicated in the tables.

The individual doses of digitoxin (Boehringer), digitoxigenin (K & K Laboratories), gitalin (Schering) and proscillaridin (Knoll) were administered in 1 ml. of water, strophanthin K (Brickman) and ouabain (Eli Lilly) in 0·2 ml. of water,

TABLE 1. *Effect of spironolactone on the toxicity of digitalis compounds*

Group	Treatment	Dose (mg)	Neuromuscular disturbances (scale: 0–3) Control	Spironolactone	Time of reading	Mortality (%) Control	Spironolactone
1–2	Digitoxin	2·0 p.o. once	1·8±0·36	0***	48 hr	30	0
3–4	Strophanthin K	0·8 s.c. once	1·8±0·15	1·5±0·31 N.S.	30 min	10	0
5–6	Gitalin	4·0 p.o. twice daily	2·2±0·48	0·1±0·1***	48 hr	90	0
7–8	Proscillaridin	5·0 p.o. twice daily	1·2±0·13	0·3±0·24**	48 hr	90	10
9–10	Digoxin	3·0 i.p. once daily	2·0±0·17	0·5±0·27***	48 hr	50	10
11–12	Ouabain	2·5 s.c. once daily	1·7±0·15	1·5±0·22 N.S.	49 hr	0	0
13–14	Digitalin (powder)	3·0 p.o. twice first day, once second day	3·0	0***	72 hr	100	0
15–16	Digitalin (solution)	0·3 i.v. once	2·8±0·13	0·8±0·25***	4 hr	0	0
17–18	Digitoxigenin	0·1 i.v. once	2·3±0·47	2·7±0·24 N.S.	30 min	30	10

The significance of the differences between the control values and those of the treated animals is expressed as follows: * $P<0·01$; ** $P<0·005$; *** $P<0·001$; N.S., not significant.

TABLE 2. *Effect of norbolethone on the toxicity of digitalis compounds*

Group	Treatment	Dose (mg)	Neuromuscular disturbances (scale: 0–3) Control	Norbolethone	Time of reading	Mortality (%) Control	Norbolethone
1–2	Digitoxin	2·0 p.o. once	2·7±0·18	0·1±0·1***	48 hr	60	0
3–4	Strophanthin K	0·8 s.c. once	1·5±0·17	1·6±0·16 N.S.	30 min	0	0
5–6	Gitalin	4·0 p.o. twice daily	2·4±0·40	0·5±0·31**	24 hr	100	10
7–8	Proscillaridin	5·0 p.o. twice daily	1·6±0·27	0·1±0·1***	48 hr	90	10
9–10	Digoxin	3·0 i.p. once daily	1·6±0·24	0·5±0·22*	48 hr	60	10
11–12	Ouabain	2·5 s.c. once daily	1·3±0·17	0·7±0·15 N.S.	49 hr	20	10
13–14	Digitalin (powder)	3·0 p.o. twice first day, once second day	3·0	1·1±0·39**	72 hr	100	10
15–16	Digitalin (solution)	0·3 i.v. once	2·8±0·13	0·8±0·13***	4 hr	0	0
17–18	Digitoxigenin	0·1 i.v. once	2·2±0·15	1·4±0·27 N.S.	30 min	10	0

The significance of the differences between the control values and those of the treated animals is expressed as follows: * $P<0·01$; ** $P<0·005$; *** $P<0·001$; N.S., not significant.

digitalin (solution in 0·3 ml. of water for intravenous and powder suspension in 1 ml. of water for oral use ; Nativelle) and digoxin (Burroughs Wellcome) in 0·3 ml. of dimethyl sulphoxide (DMSO).

Spironolactone (Aldactone ; Searle) and norbolethone (Genabol ; Wyeth) were given at the dose of 10 mg in 1 ml. of water by stomach tube, twice daily beginning on the third day with spironolactone and on the fifth day with norbolethone before the initiation of digitalis treatment and throughout the period of observation. It had been shown previously that this period of pretreatment is necessary to obtain perfect inhibition of the toxicity of digitoxin itself (Selye *et al.*, 1969). In each of the experiments ten control rats were treated with one of the digitalis compounds, and ten received, in addition, either spironolactone or norbolethone.

Throughout the experiments, the rats were maintained exclusively on Purina Laboratory Chow (Ralston Purina Company of Canada) and tap water. The convulsive motor disturbances typical of digitalis intoxication were assessed in terms of an arbitrary scale in which 0 = no change, + = uncertain gait, + + = spastic contractions of the skeletal muscles but the animal maintains upright position, + + + = intensive convulsions with the animal lying on its side. The animals of groups 1, 2, 3 and 4 were killed on the fifth day ; those of groups 5–12 on the sixth day ; those of groups 13 and 14 on the third day, and those of groups 15–18 6 hr after initiation of digitalis treatment, because of the differences in the speed with which the syndrome of intoxication developed. The percentage mortality rate listed in the tables corresponds to the number of animals that had succumbed by the time the experiment was terminated. The tables list the mean severity of the neuromotor disturbances (at the time of their maximal development in the controls) with their standard errors. The significance of the results was determined by Student's *t* test. Only when one of the two results to be compared was " 0 " did we estimate the significance of the differences on the basis of the confidence limits.

Results

As shown in Table 1, spironolactone very significantly diminished, or even abolished, the neuromotor disturbances produced by digitoxin, gitalin, proscillaridin, digoxin and both digitalin preparations, but did not significantly influence the convulsions elicited by strophanthin K, ouabain and digitoxigenin. The protection against mortality roughly paralleled the inhibition of neuromuscular disturbances.

As indicated in Table 2, norbolethone gave essentially similar results.

Discussion

It is evident that both spironolactone and norbolethone can inhibit the toxicity not only of digitoxin but also of several other digitalis preparations irrespective of the route of administration and the speed of action. It is not clear why the effect of strophanthin K, ouabain and digitoxigenin were not similarly antagonized by either of the two protective steroids, despite the similarity of both the chemical structure of these compounds and the clinical manifestations of intoxication that they produce. Of course, it must be kept in mind that, although a protective effect definitely proves an antagonistic interaction between the two drugs, the lack of protection in our experimental arrangement does not necessarily exclude it ; it is possible that the

toxic effects of strophanthin K, ouabain and digitoxigenin might also have been inhibited by the protective steroids had these been administered at different doses or at different time intervals. Yet, we may conclude that: (1) apart from digitoxin, several other related compounds are inhibited by spironolactone and norbolethone; (2) not all digitalis compounds are equally susceptible to this type of inhibition; (3) the same digitalis compounds are subject to blockade by both these protective steroids despite the otherwise very different pharmacological effects of the latter.

This work was supported by the Ministère de la Santé, Québec, the Medical Research Council of Canada (Block Term Grant MT-1829), and the Quebec Heart Foundation, Montreal.

The authors wish to thank the following companies for kindly supplying the compounds used in these experiments: Burroughs Wellcome & Co. (digoxin), Eli Lilly & Co. Ltd. (ouabain), John Wyeth & Bro. Ltd. (norbolethone), Knoll Pharmaceutical Co. (proscillaridin), Laboratoire Nativelle, Paris (digitalin), Schering Corporation Ltd. (gitalin) and Searle Co. (spironolactone, Aldactone).

REFERENCES

KAGAWA, C. M. (1964). Action of antialdosterone compounds in the laboratory. In Martini, L. & Pecile, A., *Hormonal Steroids : Biochemistry, Pharmacology and Therapeutics*, vol. 1, pp. 445–456. New York, London: Academic Press.

SELYE, H., JELINEK, J. & KRAJNY, M. (1969). Prevention of digitoxin poisoning by various steroids. *J. Pharm. Sci.*, in the Press.

(*Received June* 9, 1969)