Brit. J. Pharmacol. (1959), **14**, 83.

EFFECT OF VARIOUS ELECTROLYTES UPON CARDIAC AND SKELETAL MUSCULATURE

BY

H. SELYE AND E. BAJUSZ

From the Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada

(RECEIVED OCTOBER 27, 1958)

In rats kept on a low-potassium diet that contains only maintenance levels of magnesium, cardiac necroses and muscular cramps were readily induced by the oral administration of sodium perchlorate or disodium hydrogen phosphate. The precipitation of these cardiac and skeletal muscle changes by sodium chlorate was prevented by the prophylactic administration of either potassium or magnesium chlorides. The protective effect of these chlorides against the cardiotoxic and convulsive effects of disodium hydrogen phosphate has already been demonstrated by our earlier experiments. Sodium sulphate produced cardiac necroses in rats maintained on the same diet, and both potassium and magnesium chlorides had a prophylactic action. Unlike sodium perchlorate, however, sodium sulphate produced no muscular cramps under these conditions. Equimolecular amounts of sodium given in the form of sodium chloride (instead of sodium perchlorate, sodium sulphate, or disodium hydrogen phosphate) did not cause cardiac necroses or muscular cramps in rats maintained on the potassium-deficient diet. As the same three sodium salts, namely the perchlorate, the sulphate, and the hydrogen phosphate, produced cardiac necroses in rats sensitized by either a potassium-deficient diet or by certain corticoids, it seems that the anion must play a decisive rôle, since equivalent amounts of NaCl are ineffective.

Our investigations on the electrolyte-steroid-cardiopathy which is characterized by massive myocardial necroses (referred to subsequently as the cardiopathy) revealed that, in the corticoid-conditioned rat, the toxicity of sodium can be decisively influenced both by the anion with which sodium is associated and by the concurrent administration of various other cations. For example, in rats suitably conditioned with certain corticoids, it is possible to produce extensive myocardial necroses and fatal cardiac failure with Na_2HPO_4, NaH_2PO_4, $NaClO_4$, or Na_2SO_4, while equimolecular amounts of NaCl are ineffective. It has also been observed that the cardiopathy thus elicited by corticoids with sensitizing sodium salts is completely prevented by the simultaneous administration of $MgCl_2$ or KCl (Selye, 1958).

The importance of potassium and magnesium for the maintenance of normal cardiac structure is further substantiated by the numerous observations that rats kept on potassium-deficient (Follis, 1942; Schrader, Puckett, and Salmon, 1937; Thomas, Mylon, and Winternitz, 1940) or magnesium-deficient (Greenberg, Anderson, and Tufts, 1936; Lowenhaupt, Schulman, and Greenberg, 1950; Schrader *et al.*, 1937) diets tend to develop focal myocardial necroses. In magnesium-deficient animals, these lesions are frequently accompanied by convulsions. Subsequent studies revealed that, in rats maintained on a low-potassium diet that contained only maintenance levels of magnesium cardiac necroses developed, but the necroses could be prevented by the administration of KCl and $MgCl_2$. On the other hand, before this diet in itself produced any obvious morbid changes, Na_2HPO_4 supplements (unlike equivalent amounts of NaCl) rapidly provoke the development of severe cardiac necroses and muscular cramps; but even these can be prevented by KCl or $MgCl_2$. Obviously, Mg^{++} and HPO_4'' are of importance in the development of the syndrome usually ascribed simply to potassium deficiency, and, under certain conditions, magnesium and potassium can substitute for each other (Selye and Bajusz, 1958).

$NaClO_4$ and Na_2SO_4, like Na_2HPO_4, were highly effective in producing a cardiopathy in corticoid-conditioned rats. Hence, it seemed of interest to determine whether the perchlorate and dibasic sulphate of sodium would also be especially

toxic to animals maintained on a potassium-deficient diet and, if so, whether their toxicity could be counteracted by KCl and $MgCl_2$.

Materials and Methods

Ninety female Sprague-Dawley rats, with an initial mean body-weight of 50 g. (range, 46 to 56 g.), were placed on the low-potassium diet of the Nutritional Biochemicals Corporation (Cleveland, Ohio) for 8 days. This diet consists of: maize starch 64.2%; casein 30%; butterfat 3.5%; $CaCO_3$ 1.3%; NaCl 1%; and a vitamin mixture which provided in each 100 lb. of diet: vitamin A concentrate (200,000 units/g.) 4.5 g.; vitamin D concentrate (400,000 units/g.) 0.25 g.; α-tocopherol 5.0 g.; ascorbic acid 45.0 g.; inositol 5.0 g.; choline chloride 75.0 g.; menaphthone 2.25 g.; *p*-aminobenzoic acid 5.0 g.; nicotinic acid 4.5 g.; riboflavin 1.0 g.; pyridoxine hydrochloride 1.0 g.; aneurine hydrochloride 1.0 g.; calcium pantothenate 3.0 g.; biotin 20.0 g.; folic acid 90.0 mg.; vitamin B_{12} 1.35 mg. On the 9th day, the rats were subdivided into 9 equal groups and treated as indicated in Table I. $NaClO_4.H_2O$ (Fisher), KCl (Fisher), $MgCl_2.6H_2O$ (Merck), Na_2SO_4 (Merck), and Na_2HPO_4 (Fisher), all "Analytical Reagent" degree of purity, were administered at the dose of 0.5 m.mole, and NaCl (Brickman) at 1 m.mole, in 2 ml. of water, at 10 a.m. and 4 p.m., by stomach tube. In the groups which received two of these salts simultaneously, 0.5 m.mole of each was administered conjointly in 2 ml. of water, in order to keep the fluid volume constant.

TABLE I

EFFECT OF VARIOUS ELECTROLYTES UPON CARDIAC AND SKELETAL MUSCULATURE

The 10 animals of each group were kept on the potassium-deficient diet described in the text. The means and standard errors of the necrosis and cramp scores are expressed in an arbitrary scale of 0 to 3.

Group	Treatment	Cardiac Necrosis	Muscular Cramps	Mortality (%)
I	None	0·1±0·1	0	0
II	$NaClO_4$	1·7±0·30	3·0±0	0
III	$NaClO_4$+KCl	0	0	0
IV	$NaClO_4$+$MgCl_2$	0·4±0·30	1·1±0·23	30
V	Na_2SO_4	1·1±0·40	0	0
VI	Na_2SO_4+KCl	0·1±0·1	0	0
VII	Na_2SO_4+$MgCl_2$	0·5±0·28	0	50
VIII	NaCl	0·1±0·1	0	0
IX	Na_2HPO_4	1·2±0·25	3·0±0	50

Muscular cramps were noted soon after the administration of $NaClO_4$ (Group II) and Na_2HPO_4 (Group IX), but the regular test for these cramps was performed one hour following the second gavage, that is, at 5 p.m. on the 9th day. The "Flick Test" was used for this purpose, because it proved to be highly effective in revealing a latent tendency for muscular cramps. The test consisted in placing the rat on a flat surface (such as a table) and giving it a flick on the sacral region with the index finger. Normal rats either showed no response to this slight irritation or took a few steps forward, while animals with a marked tendency to develop cramps (for instance, after heavy overdosage with $NaClO_4$) made spastic extensor movements with their hind paws. The results of this test were expressed in an arbitrary scale running from 0 to 3, in which 0 meant no spastic response; 1, brief extensor cramp which disappeared immediately; 2, prolonged extensor cramp; 3, spreading of the cramp to the whole body with a characteristic hunching of the back and extension caudally of the forelimbs. This peculiar muscular response was particularly characteristic of animals receiving $NaClO_4$. Occasionally, it also occurred after heavy overdosage with Na_2HPO_4, but the latter salt tended to cause a much more generalized tetanic state with tremor; this, incidentally, was similarly precipitated by the flick test. At none of the concentrations of Na_2SO_4 used did animals so treated give a response to the flick test.

If very large amounts of $NaClO_4$ or Na_2HPO_4 were given, muscular cramps occurred even in rats kept on normal diets. For the interpretation of our results it is important to note, therefore, that in a preliminary experiment on three groups of ten normal rats, similar to those described in Table I but kept on "Purina Fox Chow," the flick test was completely negative after treatment with comparable doses of $NaClO_4$, Na_2SO_4 and Na_2HPO_4, respectively.

The main experiment was terminated on the 10th day, that is, one day after the electrolyte treatment ceased. The hearts were fixed in neutral formalin and stained with the acid-fuchsin technique (Selye, 1958) for the demonstration of early prenecrotic changes, and with the silver nitrate technique of von Kossa for the histochemical detection of calcium. The intensity of the cardiac necroses was assessed in terms of an arbitrary scale of 0 to 3. The means of these readings, as well as those of the flick test, are listed (with standard errors) in Table I.

Results

Table I indicates that, following such a brief period on the potassium-deficient diet, almost no cardiac necrosis occurred in the control animals (Group I). On the other hand, a single day of treatment with either $NaClO_4$ (Group II), Na_2SO_4 (Group V) or Na_2HPO_4 (Group IX) sufficed to cause widespread cardiac necroses in the majority of the experimental animals. These necroses are virtually indistinguishable from those of the cardiopathy that have been described elsewhere (Selye, 1958). They were characterized by a widespread distribution, with definite predilection for the subendocardial layers of both ventricles and for the entire thickness of the right ventricle. In the initial stages, the affected muscle fibres stained deeply and selectively with our fuchsin technique, but later they were absorbed and replaced by histiocytes and connective tissue cells. At this

stage, individual fuchsinophilic fibres might still be seen in the immediate vicinity of the inflammatory foci. As shown by von Kossa's stain, the necrotic muscle fibres often, but not always, underwent calcification.

This cardiotoxic effect of $NaClO_4$ could be prevented or at least diminished by KCl (Group III) and $MgCl_2$ (Group IV). Na_2SO_4 was somewhat (but not significantly) less effective than $NaClO_4$ in producing these lesions; but here again KCl (Group VI) and to a lesser extent $MgCl_2$ (Group VII) effectively prevented the damage. Both the aggravation produced by $NaClO_4$ and Na_2SO_4, and the prevention of this by KCl and $MgCl_2$, were statistically highly significant in all instances ($P<0.01$). Only the prophylactic effect of $MgCl_2$ against damage by Na_2SO_4 was not statistically significant.

In view of the possible rôle played by sodium in the precipitation of this cardiac necrosis, it was interesting that NaCl proved totally ineffective in this respect. This was all the more significant as NaCl was given at a dose level of 2 m.mole, twice daily, so that the amount of sodium administered in this form was equivalent to that of the Na_2SO_4 and Na_2HPO_4 and to twice that of the $NaClO_4$; yet the three last-mentioned substances proved effective under the same circumstances.

The last group (IX), in which Na_2HPO_4 was given, merely served to confirm our earlier work on the toxicity of this electrolyte to rats maintained on our potassium-deficient diet, and we noted that, as expected, the salt induced severe cardiac necroses.

As to the production of the muscular cramps the results were very similar: $NaClO_4$ and Na_2HPO_4 produced intense muscular cramps when given alone. As with Na_2HPO_4 (Selye and Bajusz, 1958), this effect of $NaClO_4$ could be inhibited by KCl and to a lesser, but still highly significant, extent by $MgCl_2$. On the other hand, Na_2SO_4, like NaCl, elicited no neuromuscular disturbances.

In this short-term experiment, treatment with $NaClO_4$, Na_2SO_4 or NaCl during one day caused no mortality, while Na_2HPO_4 resulted in the death of half our animals. Among the chlorides prophylactically effective, KCl appeared to be preferable under these conditions, in that it not only inhibited the cardiac necroses and muscular cramps completely, but caused no mortality. $MgCl_2$ was not only somewhat less effective in regard to the prevention of cardiac and muscular lesions, but it induced a comparatively high mortality.

Discussion

The most interesting outcome of these investigations appears to us to be that the sodium salts ($NaClO_4$, Na_2SO_4, Na_2HPO_4) previously shown to produce cardiac necroses in rats which had received corticoids have the same effect in animals on a potassium-deficient diet. This similarity in the conditioning influence of potassium deficiency and of corticoid overdosage is emphasized by the fact that equivalent amounts of sodium given in the form of NaCl are ineffective in both these circumstances. Further, KCl and $MgCl_2$, which prevent the cardiac necroses produced by sensitizing sodium salts in corticoid-treated rats, exert this same protective effect in animals treated with sensitizing sodium salts while on a potassium-deficient diet. At least, in the case of the cardiopathy, the sensitization must be due to sodium, since all the salts of cations other than sodium consistently proved ineffective in producing cardiac necroses after corticoid treatment (Selye, 1958); yet the anion also plays a decisive rôle, since NaCl was ineffective, both after corticoid-conditioning and during potassium deficiency.

It is also interesting that in the experiments reported here, as in those concerning the cardiopathy (Selye, 1958), $MgCl_2$ can replace KCl as a prophylactic agent. The low-potassium diet which we used contained 52 mg./kg. of magnesium, which corresponded to the minimal amount necessary for growth according to Tufts and Greenberg (1938). However, even if we assume that our diet was simultaneously deficient in potassium and magnesium, it is difficult to understand why it should predispose to the production of cardiac necroses and muscular cramps by $NaClO_4$, Na_2SO_4, and Na_2HPO_4 but not by NaCl, and why the predisposition so induced should be prevented by either KCl or $MgCl_2$. It would be tempting to assume that the cardiac necroses are, in all instances, hypokalaemic. In this event, the sodium salts could act because of the well-known antagonism between sodium and potassium which has been demonstrated in many other test-objects. However, the pronounced hypokalaemia which is induced in rats by heavy overdosage with various mineralocorticoids produces neither cardiac necroses nor muscular cramps (Selye, 1958); indeed, in the monkey, the dog and man (but not the rat), such hypokalaemia is accompanied by flaccid muscular paralysis (Selye, 1950). It is also noteworthy that, in the present experimental series, even the animals which died with grade 3 cardiac necroses due to the Na_2SO_4 treatment

never showed any trace of muscular cramps; hence, the effect of sensitizing sodium salts upon cardiac and skeletal muscle cannot be inseparably interrelated. To this we may add that, in our previous studies on the effect of Na_2HPO_4 upon rats kept on this same potassium deficient diet, there developed, in addition to the cardiac necroses and muscular cramps, an intense nephrocalcinosis which was also readily prevented by either $MgCl_2$ or KCl (Selye and Bajusz, 1958). Apparently, in this respect magnesium can also substitute for potassium.

In view of all these findings, it is not possible to explain our observations by the over-simplified theory that the diet predisposes to the hypokalaemia, and that all the manifestations precipitated by the sensitizing sodium salts are merely the consequences of an aggravation of potassium deficiency by an excess of sodium. Further investigations will be necessary to elucidate the mechanism of these complex ionic interactions, but it is already evident that the conditioning effect of certain corticoids can be rather closely reproduced by an experimental dietary insufficiency.

These investigations were supported by grants from The Muscular Dystrophy Association of Canada and the Quebec Asbestos Mining Association.

References

Follis, R. H. (1942). *Bull. Johns Hopk. Hosp.*, **71**, 235.
Greenberg, D. M., Anderson, C. E., and Tufts, E. V. (1936). *J. biol. Chem.*, **114**, xliii.
Lowenhaupt, E., Schulman, M. P., and Greenberg, D. M. (1950). *A.M.A. Arch. Path.*, **49**, 427.
Schrader, G. A., Prickett, C. O., and Salmon, W. D. (1937). *J. Nutr.*, **14**, 85.
Selye, H. (1950). *The Physiology and Pathology of Exposure to Stress.* Montreal: Acta, Inc., Med. Pub., Montreal.
—— (1958). *The Chemical Prevention of Cardiac Necroses.* New York: Ronald Press.
—— and Bajusz, E. (1958). *Proc. Soc. exp. Biol. (N.Y.)*, **98**, 580.
Thomas, R. M., Mylon, E., and Winternitz, M. C. (1940). *Yale J. Biol. Med.*, **12**, 345.
Tufts, E. V., and Greenberg, D. M. (1938). *J. biol. Chem.*, **122**, 715.