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RELEASE OF CREATINE PHOSPHOKINASE FROM MUSCLE—II

EFFECT OF MULTIPLE DOSES OF POLYMYXIN B OR COMPOUND 48/80 ON INCREASES IN PLASMA ENZYME LEVELS

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Abstract—A single dose of Polymyxin B, 5 mg/kg, or Compound 48/80, 3 mg/kg, increases the skeletal muscle-type creatine phosphokinase (PCPK) activity in rat plasma.[3] After a 1- to 3-day period of daily treatments with increasing doses of Polymyxin B or Compound 48/80, the same doses of Polymyxin B or Compound 48/80, i.p., as cited above produce markedly smaller or no increases in PCPK, plasma aspartate aminotransferase (GOT) or plasma lactic dehydrogenase activities. However, administration of Polymyxin B, 10 mg/kg, i.p., or Compound 48/80, 6 mg/kg, i.p., on the day after completion of the 3-day pretreatment with the same drug did produce increased PCPK activities. The increase in PCPK activity produced by Polymyxin B, 5 mg/kg, or Compound 48/80, 3 mg/kg, was significantly smaller in rats pretreated with Compound 48/80 or Polymyxin B, respectively, for 3 days, than in naive rats. Ten days after completing the 3-day Polymyxin B pretreatment, Polymyxin B, 5 mg/kg, produced an increase in PCPK activities. The increases in PCPK activity produced by restraint stress or other pharmacological agents were not diminished by prior treatment with Polymyxin B for 3 days. The evidence suggests that tolerance to the toxic effects of Polymyxin B or Compound 48/80 on skeletal muscle and other tissues develop with two or more doses of Polymyxin B or Compound 48/80.

A SINGLE dose of Polymyxin B or Compound 48/80, both of which are well known to disrupt mast cells,[1,2] produces marked increases in the plasma activities of the skeletal muscle isoenzyme of creatine phosphokinase (CPK, EC 2.7.3.2) in rats.[3] This was believed to be due to toxic effects of Polymyxin B and Compound 48/80 on skeletal muscle. In a subsequent study, Polymyxin B and Compound 48/80 were found to produce necrosis of skeletal muscle in the rat as well as more subtle pathological changes in the myofibrils and Z-band of some surviving fibers.*

We wish to report that pretreatment with two or more doses of Polymyxin B, or Compound 48/80, significantly inhibits the increase in skeletal muscle-type creatine phosphokinase (PCPK) activity produced by Polymyxin B, 5 mg/kg, or Compound 48/80, 3 mg/kg, respectively. However, pretreatment with multiple doses of Polymyxin B or Compound 48/80 does not diminish the increases in PCPK activity produced in naive rats by: (1) restraint at 2° (cold restraint, CR);[4] (2) phencyclidine (Phen) and restraint at room temperature (RRT);[5] or (3) chlorpromazine (Cpz).[6] It is proposed that decreased CPK release from skeletal muscle produced by Polymyxin B after pretreatment with multiple doses of Polymyxin B or Compound 48/80 is the result of the development of tolerance to the toxic effects of these drugs.

* H. Meltzer, E. McBride and G. McWhorter, unpublished data.

MATERIALS AND METHODS

Male Sprague–Dawley rats, purchased from Sprague–Dawley, Inc., Madison, Wis., weighing 140–150 g, were used throughout these experiments. They were maintained in a temperature-controlled room at 22° and given Purina rat pellets and water *ad lib.*

Polymyxin B was administered i.p. according to a modification of the schedule of Parratt and West: day 1, 5 mg/kg in the morning; day 2, 5 mg/kg in the morning and 6 hr later; day 3, 7·5 mg/kg in the morning and 6 hr later.[7] Compound 48/80 was administered i.p. according to the schedule of Parratt and West: day 1, 1 mg/kg in the morning, 2 mg/kg 6 hr later; day 2, 2 mg/kg in the morning, 3 mg/kg 6 hr later; day 3, 4 mg/kg in the morning, 5 mg/kg 6 hr later.[7] These 3-day schedules for administering Polymyxin B and Compound 48/80 will be referred to as the 3-day standard schedules. Control rats were given an equal volume of isotonic saline according to the same time schedule. In some studies, only part of the 3-day standard schedules of Polymyxin B were administered or different doses of Polymyxin B were administered at the same time period as the standard schedule.

On the day after the completion of the 3-day standard schedule of Polymyxin B, Compound 48/80 or saline, or some part thereof, groups of six rats were given: (1) Polymyxin B, various doses, i.p.; (2) Compound 48/80, various doses, i.p.; (3) Phen, 5 mg/kg, i.p., or saline, 15 min before 2 hr of RRT or CR; (4) Cpz, 25 mg/kg, i.p. All drugs were dissolved in isotonic saline and 0·1 ml was injected. Rats were restrained according to the method of Senay and Levine.[8]

Methods of blood collection, temperature determination and determination of CPK, lactic dehydrogenase (LDH) and aspartate aminotransferase (GOT) activities in plasma have been previously described.[3,4] Plasma was frozen for assay within 48 hr of collection.

Polymyxin B sulfate was a gift of Pfizer Laboratories, New York, N.Y. Compound 48/80 was a gift of Burroughs Wellcome, Tuckahoe, N.Y.; phencyclidine (Sernalyn) was purchased from Bio-Ceutics, Inc., St. Joseph, Mo. Chlorpromazine hydrochloride was a gift of Smith, Kline & French Laboratories, Philadelphia, Pa. The Polymyxin B, Compound 48/80 and Cpz were dissolved in saline for administration.

All results are expressed as mean ± S.E.M. The significance of the difference between two means was determined by means of an unpaired *t*-test with correction for unequal variances where appropriate.[9]

RESULTS

Effect of standard 3-day pretreatment with Polymyxin B or Compound 48/80 on plasma enzyme activities. Results are given in Table 1. Polymyxin B, 5 mg/kg (group II), or Compound 48/80, 3 mg/kg (group V), significantly raised PCPK, GOT and LDH activities 90 min after injection. Eighteen hr after the last injection of the 3-day standard course of Polymyxin B (group III) or Compound 48/80 (group VI), PCPK, GOT and LDH activities were not significantly different from control levels. Injections of Polymyxin B, 5 mg/kg (group IV) or Compound 48/80, 3 mg/kg (group VII), 18 hr after treatment with the standard 3-day course of Polymyxin B or Compound 48/80, respectively, did not increase PCPK or GOT activities, but did very slightly raise LDH activity. There was also no increase in PCPK activity of group IV rats sacrificed at 4, 7 or 24 hr after Polymyxin B on day 4 (data not presented).

TABLE 1. EFFECT OF STANDARD SCHEDULE OF POLYMYXIN B OR COMPOUND 48/80 ON INCREASE IN PLASMA ENZYME LEVELS*

Group	Pretreatment	Treatment	Dose (mg/kg)	Time of sacrifice (hr)	PCPK (mU/ml)	GOT (mU/ml)	LDH (mU/ml)
I	None	Saline		1·5	61 ± 2 (36)†	35 ± 3 (16)	21 ± 3 (14)
II	None	Polymyxin B	5	1·5	570 ± 20 (50)‡	117 ± 10 (6)‡	715 ± 106 (6)‡
III	Polymyxin B, 3-day§	Saline		1·5	71 ± 3 (6)‖	36 ± 4 (6)‖	28 ± 5 (6)‖
IV	Polymyxin B, 3-day	Polymyxin B	5	1·5	78 ± 3 (15)‖	36 ± 6 (6)‖	44 ± 5 (6)‡
V	None	Compound 48/80	3	2	320 ± 33 (28)‡	95 ± 13 (6)‡	456 ± 96 (6)‡
VI	Compound 48/80, 3-day	Saline		2	62 ± 3 (6)‖	20 ± 2 (6)¶	28 ± 4 (6)‖
VII	Compound 48/80, 3-day	Compound 48/80	3	2	68 ± 4 (6)‖	24 ± 3 (6)‖	32 ± 2 (6)**

* The indicated treatment was given 18 hr after end of the pretreatment. Animals were sacrificed 1·5 hr or 2 hr after the treatment, as indicated.
† Number of rats in parentheses.
‡ Significantly different from control levels, $P < 0·001$.
§ Three-day refers to the standard schedules described in Methods section.
‖ Not significantly different from control levels.
¶ Significantly different from control levels, $P < 0·01$.
** Significantly different from control levels, $P < 0·005$.

Ten days after completing the 3-day standard schedule of Polymyxin B pretreatment, Polymyxin B, 5 mg/kg, produced an increase in PCPK activities to 416 mU/ml ± 62 mU/ml (N = 6). This is significantly less than the PCPK activity in naive rats given the same dose of Polymyxin B but greater than the increase in PCPK activity produced by Polymyxin B immediately after the 3-day standard schedule.

Effect of larger doses of Polymyxin B and Compound 48/80 *on PCPK levels in rats pretreated with increasing doses of either drug.* To determine if tolerance to the effects of Polymyxin B and Compound 48/80 on PCPK activity had developed as a consequence of the repeated increasing dosages of either drug during the standard 3-day course of Polymyxin B or Compound 48/80, rats were challenged with higher doses of either drug on day 4. As can be seen in Table 2, PCPK activity did increase after treatment with higher dosages of Polymyxin B or Compound 48/80. The dose of Compound 48/80 used would have been lethal to naive rats. (The LD_{50} in naive rats for Compound 48/80 is 5 mg/kg.)[10] However, the increase in PCPK activity 90 min after 10 mg/kg of Polymyxin B in rats pretreated with the 3-day standard schedule of Polymyxin B was significantly less than that produced by the same dose in naive rats, 1554 mU/ml ± 159 mU/ml (N = 6), $P < 0{\cdot}001$. The effects of a 10 mg/kg dose of Polymyxin B on PCPK activity could be completely inhibited if the rats were pretreated with Polymyxin B according to the following schedule: (1) day 1, 5 mg/kg, a.m.; day 2, 7·5 mg/kg, a.m., 10 mg/kg, 6 hr later; day 3, 12·5 mg/kg, a.m., 12·5 mg/kg, 6 hr later.

TABLE 2. EFFECT OF PRETREATMENT WITH POLYMYXIN B OR COMPOUND 48/80 ON INCREASE IN PCPK LEVELS AFTER VARIOUS DOSES OF POLYMYXIN B OR COMPOUND 48/80*

Pretreatment	Treatment	Dose (mg/kg, i.p.)	Survivors	PCPK levels (mU/ml)†
Polymyxin B, 3-day	Polymyxin B	5	11/11	73 ± 3
Polymyxin B, 3-day	Polymyxin B	10	14/14	243 ± 24‡
Polymyxin B, 3-day	Polymyxin B	12·5	4/4	553 ± 128‡
Polymyxin B, 3-day	Polymyxin B	15	0/4	
Compound 48/80, 3-day	Compound 48/80	3	6/6	44 ± 4
Compound 48/80, 3-day	Compound 48/80	6	7/7	149 ± 13§
Compound 48/80, 3-day	Compound 48/80	8	0/4	

* All rats received the treatment injection of Polymyxin B or Compound 48/80 on the day after completion of pretreatment.

† Mean ± S.E.M.

‡ Significantly greater than effects of Polymyxin B, 5 mg/kg ($P > 0{\cdot}001$).

§ Significantly greater than effects of Compound 48/80, 3 mg/kg ($P < 0{\cdot}001$).

Other aspects of the rats' response to Polymyxin B were not as inhibited by pretreatment with the 3-day standard schedule as was the increase in PCPK activity. The mean fall in temperature 90 min after Polymyxin B, 5 mg/kg, in twelve rats pretreated with the 3-day standard schedule of Polymyxin B was 1·0° ± 0·1° which is significantly less than that of twenty naive rats who received the same dose of Polymyxin B, 1·4° ± 0·1° ($P < 0{\cdot}001$).[3] Furthermore, the 5 mg/kg dose of Polymyxin B on the day after the standard schedule of Polymyxin B still produced erythema, slight edema and marked vasodilatation in most rats, as previously noted by Parratt and West.[7]

Effect of pretreatment with Polymyxin B or Compound 48/80 *on increase in PCPK activity produced by CR, Phen and RRT, and Cpz.* It was of interest to determine if the standard 3-day schedule of treatment with Polymyxin B or Compound 48/80 blocked the increase in PCPK activity produced by other means. Such studies might help decide between development of tolerance, increased clearance of CPK and other enzymes from serum, or inability of enzymes from damaged muscle or other tissues to enter the circulation as causes of the failure of the final dose of Polymyxin B or Compound 48/80 to increase the activities of CPK, GOT and LDH in plasma. As can be seen from Table 3, pretreatment with Polymyxin B or Compound 48/80 did not affect the increase in PCPK activity following: (1) 2 hr of CR; (2) Phen, 5 mg/kg, i.p. 15 min before 2 hr RRT; or (3) chlorpromazine, 25 mg/kg, i.p.

TABLE 3. EFFECT OF PRETREATMENT WITH POLYMYXIN B OR COMPOUND 48/80 ON INCREASE IN PCPK LEVELS AFTER RESTRAINT STRESS OR CPZ*

Pretreatment	Treatment	PCPK levels (mU/ml)†	P
Saline	CR, 2 hr	1897 ± 449	>0·5
Polymyxin B, 3-day	CR, 2 hr	1370 ± 159	
Saline	Phen, 5 mg/kg, i.p.	1063 ± 318	>0·5
Polymyxin B, 3-day	RRT, 30 min	1301 ± 306	
Saline	Cpz, 25 mg/kg, i.p.	4780 ± 645	>0·5
Polymyxin B, 3-day	1 hr	4430 ± 720	
Saline	CR, 2 hr	1897 ± 449	>0·5
Compound 48/80, 3-day	CR, 2 hr	1497 ± 239	

* All groups consisted of six rats and received increasing doses of Polymyxin B, Compound 48/80 or saline according to the schedule cited under Methods. They were subjected to stress or drugs the morning after completion of the pretreatment schedule and sacrificed immediately after restraint or 1 hr after Cpz.

† Mean ± S.E.M.

Other studies on tolerance. To determine if the complete standard 3-day course of Polymyxin B was necessary to inhibit the increase in PCPK activity following a subsequent dose of Polymyxin B, 5 mg/kg, groups of six rats were given part of the standard 3-day course of increasing dosages of Polymyxin B prior to the challenge dose of Polymyxin B, 5 mg/kg, the day after the last previous dose. PCPK activity after the last dose was significantly less than that in naive rats only when there had been at least two preceding injections of Polymyxin B (data not presented). Resistance to the effects of Polymyxin B on PCPK activity was apparent in rats pretreated with the standard 3-day schedule of Compound 48/80. Similarly, pretreatment with the 3-day standard schedule of Polymyxin B blocked the usual effects of a 3 mg/kg dose of Compound 48/80 (data not presented).

DISCUSSION

The complete or almost complete inhibition of the increase in PCPK, GOT and LDH activities usually produced by Polymyxin B, 5 mg/kg, or Compound 48/80, 3 mg/kg,

by pretreatment with increasing doses of either drug for 3 days could represent the development of tolerance to the toxic effects of Polymyxin B on muscle. Before this hypothesis can be accepted, a number of possibilities must be considered.

A possible explanation of the inhibition of increase in PCPK activity after multiple doses of Polymyxin B or Compound 48/80 would be injury to the microcirculation or macrocirculation which might prevent enzymes released from damaged muscle from entering the circulation. That this was not the case is demonstrated by the fact that CR, Phen plus RRT, and Cpz produced increases in PCPK activity in rats pretreated with the standard 3-day course of Polymyxin B or Compound 48/80 that were not significantly different from those in naive rats. These findings also demonstrate that increased clearance of CPK (and other enzymes) from plasma could not account for the inhibition of the increase in plasma enzyme activities following Polymyxin B or Compound 48/80 on day 4 of treatment.

Delay of entry of CPK into the circulation is not likely to be the explanation of the failure of a 5 mg/kg dose of Polymyxin B to raise PCPK levels in rats pretreated with the standard 3-day schedule since no increases in PCPK activity were found in rats 4, 7 and 24 hr after the final dose of Polymyxin B.

The initial dose of Polymyxin B leads to hypotension as part of an anaphylactoid shock-like state.[11] This might produce anoxic damage of muscle and subsequent CPK release.[3] However, we have previously shown that no increase in rat PCPK activity accompanies anaphylactoid shock after treatment with dextran or ovomucoid.[3] Thus, any decrease in the extent of anaphylactoid shock after multiple doses of Polymyxin B or Compound 48/80 is not likely to be a factor in the smaller increase in PCPK activity after multiple doses of Polymyxin B or Compound 48/80.

Reite and Hausken[12] have previously shown that blood levels of Polymyxin B after i.p. injection were higher and the peak was achieved more rapidly in rats which were depleted of mast cells by Compound 48/80 than in naive rats. Thus, diminished absorption of Polymyxin B or Compound 48/80 is not likely to be the explanation of the inhibition of increase in PCPK activity after the final injection of Polymyxin B or Compound 48/80. Rather, the higher blood levels of Polymyxin B or Compound 48/80 might be expected to produce a greater degree of muscle necrosis and higher PCPK activity than found in naive rats.

The fact that the rats pretreated with the standard 3-day course of Compound 48/80 were able to survive dosages of Compound 48/80 that would be lethal to naive animals and that smaller decreases in body temperature were produced by Polymyxin B and Compound 48/80 in rats pretreated with the standard 3-day course of either drug indicates that tolerance to some effects of the drugs did develop. Diminution of the anaphylactoid shock syndrome with repeated administration of Compound 48/80 has previously been reported by Parratt and West[7] and by Dews *et al.*[10]

Also consistent with tolerance as a factor in the inhibition of the increase in PCPK activity produced by Polymyxin B, 5 mg/kg, and Compound 48/80, 3 mg/kg, in rats pretreated with the standard 3-day schedule of either agent is that Polymyxin B, 10 mg/kg, or Compound 48/80, 6 mg/kg, did raise PCPK activity in the pretreated rats (but less than naive rats). Furthermore, exposure to a 3-day schedule of Polymyxin B, which included doses as high as 12·5 mg/kg, blocked the increase in PCPK activity produced by 10 mg/kg of Polymyxin B. That Polymyxin B did not increase PCPK activity in rats pretreated with the standard 3-day schedule of Compound 48/80 and

vice versa suggests that cross-tolerance develops. The tolerance to the effects of Polymyxin B which leads to increase in PCPK levels is first manifest after the third injection of Polymyxin B, indicating that only two exposures to the drug are required to produce some tolerance. The process is essentially complete after the fourth injection. Preliminary studies of the persistence of the effect suggests that by 10 days after completion of the 3-day standard schedule, responsiveness to the toxic effects of the drug has partially returned.

It is of interest that the percentage increase in PCPK activity produced by Polymyxin B, 5 mg/kg, i.p. (830 per cent) was less than that produced in LDH activity (3300 per cent) (Table 1). The percentage increase in LDH activity produced by Compound 48/80 was also greater than the increase in PCPK activity. PCPK activity is generally the most sensitive indicator of muscle damage.[13] The large increases in LDH activity probably reflect damage to other organs rich in these enzymes and lacking CPK. Toxic effects of Polymyxin B on kidney and neural tissues are well known.[14] The blockade of the increase in GOT and LDH activities produced by the 3-day standard courses of Polymyxin B or Compound 48/80 suggests that skeletal muscle is not the only organ which becomes resistant to the effects of Polymyxin B or Compound 48/80.

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