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RAPID COMMUNICATIONS

EFFECTS OF EICOSAPENTAENOIC ACID (20:5ω3) ON STRESS REACTIVITY IN RATS

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ABSTRACT: This study examined the effects of eicosapentaenoic acid (EPA) on cardiovascular responses to isolation stress in male rats. Group-reared rats, on a fat-free diet, were given olive oil (OL), or EPA in OL (1.47×10^{-7} mol/hr) via 8 week osmctic pumps, or a dummy pump (DUM), 2 weeks prior to a 4 week isolation period. Blood pressure (BP), heart rate, and body weight were monitored weekly and pressor responses to i.a. norepinephrine and angiotensin were assessed at the end of the study. BP increased during stress in all animals vs. pre-stress conditions, but was attenuated by EPA ($p<0.001$). Heart rate also increased during stress in all groups, but was greater in the EPA group ($p<0.001$). In contrast, body weight gain during stress was similar in DUM and EPA groups, but depressed by OL ($p<0.001$). Vascular response to norepinephrine was enhanced by EPA vs. DUM and OL, whereas the response to angiotensin was similar in EPA and DUM groups, but reduced by OL. These data suggest that EPA may attenuate cardiovascular responses to psychological stress.

Dietary ω3 and ω6 fatty acids, precursors for endogenous prostanoid synthesis, are capable of altering cardiovascular function (1-3). Stress hormones have been demonstrated to inhibit the activity of Δ6- and Δ5-desaturase, the rate limiting enzymes in the metabolism of ω3 and ω6 fatty acids (4).

We have recently demonstrated that the post- Δ6-desaturase products of the ω6 series, 18:3 ω6 and 20:4 ω6, but not the pre- Δ6-desaturase compound, 18:2 ω6, are capable of attenuating the pressor response to psychological (isolation) stress. In addition, the pre- Δ6-desaturase compound of the ω3 series, 18:3ω3, was shown to be without effect (5).

A great deal of attention is currently being focussed on a post- Δ6- and Δ5-desaturase compound of the ω3 series, 20:5ω3, or eicosapentaenoic acid (EPA), for its blood pressure reducing and anti-aggregatory effects (6-7). No work has been performed, however, to assess its effectiveness in reducing the cardiovascular responses to stress.

The purpose of the present study was to examine the effects of EPA on cardiovascular reactivity to chronic psychological stress.

MATERIALS AND METHODS

Adult male Wistar Kyoto rats were purchased at 6 wks of age (Taconic Farms) and group acclimated (4/cage) for 5-7 wks. After fasting for 2 days, they were placed on a fat-free diet for the duration of the study. Two wks later, animals were divided into groups and received 8 wk osmotic pumps (Alza) i.p., releasing olive oil (OL, 1.5 µl/hr, n=5) or EPA (1.47×10^{-7} mol/hr, n=5) in OL (5). EPA released i.p. (0.4 mg/hr) enters the portal circulation, approximating entry via the oral route. A third group received a dummy pump (DUM n=6). All animals remained group housed for a 2 wk control period following surgery, and were then placed in individual cages for a 4 wk period of isolation stress (5, 8).

Systolic BP and heart rates were indirectly measured via the tail-cuff tech-

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nique (5, 8-9) wkly, as well as 24 hr prior to and following isolation. Body weight was also measured at these times. At the end of the 4 wk isolation period, animals were anesthetized with halothane and arterially cannulated. Following separate injections of an ED_{50} of norepinephrine (NOR, 4.2 µg) and angiotensin (ANG, 1.25 µg), BP and heart rate were directly monitored with a Coulbourne physiograph, in order to assess cardiovascular reactivity.

Blood pressure, heart rate, and body weight were analyzed using a 2 way analysis of covariance, in order to adjust for pretreatment baseline differences. Where a significance of $p<0.05$ was attained, specific points of difference were determined using planned orthogonal comparisons (10). Cardiovascular reactivity was analyzed using the Student 't' test.

RESULTS

Isolation significantly increased systolic BP (Fig. 1) in DUM ($p<0.01$, S2-S4), OL ($p<0.001$, SO-S4), and EPA ($p<0.001$, SO and S4; $p<0.05$, S1-S2) groups.

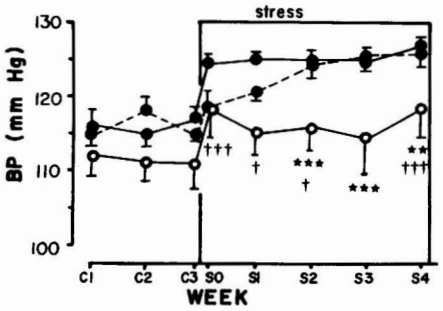

Fig. 1. Systolic BP ($\bar{x}$ ± S.E.) of adult male rats over a 2 week period before, and during a 4 week period of social isolation (stress). Measures were taken weekly, as well as 24 hr prior to (C3) and following (SO) isolation. Groups are DUM(●----●), OL(●——●), and EPA in OH (O—O). Statistical indicators based on ANOVA covaried for C1.

*p<0.05 vs. DUM and OL; **p<0.01 vs. DUM and OL; *** p<0.001 vs. DUM and OL

For EPA:† p<0.05 vs. C1; ††† p<0.001 vs. C1

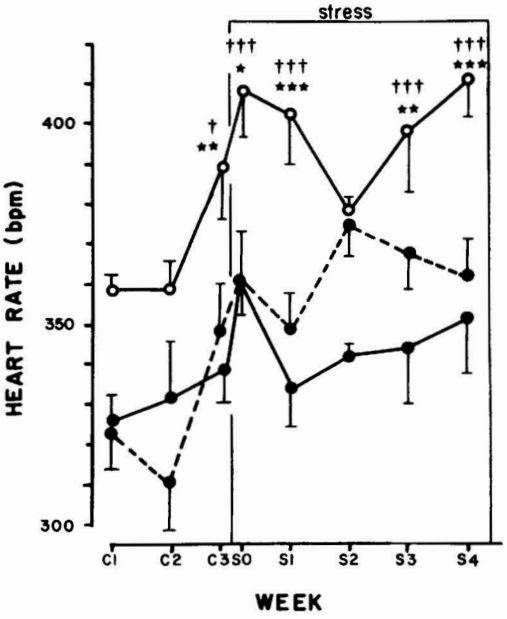

Fig. 2. Heart rate ($\bar{x}$ ∓ S.E.) of adult male rats over a 2 week period before, and during a 4 week period of social isolation (stress). Measures were taken weekly, as well as 24 hr prior to (C3) and following (SO) isolation. Groups and statistical indicators as in Fig. 1.

However, BP during isolation was significantly lower in the EPA group than in the DUM or OL groups ($p<0.001$, S2-S3; $p<0.01$, S4). Isolation increased heart rates (Fig. 2) in all groups vs pre-stress levels, although the patterns varied greatly. However, adjusted heart rate was significantly greater in the EPA group during stress, than in either DUM or OL groups ($p<0.05$, SO; $p<0.001$, S1 and S4; $p<0.01$, S3). Furthermore, pre-stress heart rates (unadjusted) were higher in the EPA group than in DUM or OL groups.

Adjusted body weight gain during stress (Table I) was similar in the DUM and EPA groups, which was greater than in the OL group ($p<0.001$, S1-S4).

Cardiovascular responses to NOR (Table II) were similar between groups, with the exception that the systolic BP response was greater in the EPA group ($p<0.05$). In contrast, EPA, reduced

Table I

	Raw Data (gm)			Adjusted Means (gm)		
	DUM	OL	EPA	DUM	OL	EPA
C1	371.8±8.3	378.0±20.2	319.0±18.5	-	-	-
C2	370.3±7.9	392.8±15.0	322.8±18.7	366.5	383.1	368.3
C3	390.3±8.1	399.5±14.1	349.1±19.7	386.4	389.9	394.5
SO	397.8±9.3	405.6±13.1	350.0±19.8	393.9	395.9	395.5
S1	419.1±8.4	392.4±11.6	366.2±20.6	415.2***	382.7	411.6***
S2	435.6±9.9	401.8±10.1	384.4±22.8	431.8***	392.1	429.8***
S3	449.4±11.1	407.2±8.8	390.4±21.8	445.6***	397.5	435.9***
S4	454.0±10.7	427.3±10.9	424.8±25.6	450.1***	417.6	470.2***

Raw ($\bar{x}$ ± S.E.) and adjusted ($\bar{x}$, covaried for C1) body weights of DUM-(n=6), OL-(n=5) and EPA in OL-(n=5) treated rats for 2 weeks prior to, and 4 weeks following social isolation stress. Measures were taken at weekly intervals during the pre-stress (C1, C2) and stress (S1, S2, S3, S4) conditions, as well as 24 hrs prior to (C3) and following (SO) isolation.
For adjusted means, ***p<0.001 vs. OL

Table II

	DUM (n=6)	OL (n=5)	EPA (n=5)	
NOR				
Duration of response (sec)	287±59	314±33	292±31	
HR response (min^{-1})	41±11	50±16	30±9	
Δs BP (mm Hg)	44± 6	40± 5	60±5	*†
Δd BP (mm Hg)	44± 6	39± 5	51± 5	
ANG				
Duration of response (sec)	217±37	287±18	152± 9	**
HR response (min^{-1})	73±29	9± 9	33±18	
Δs BP (mm Hg)	33± 4	21± 1	38± 3	***
Δd BP (mm Hg)	32± 4	21± 2	36± 3	**

Cardiovascular responses ($\bar{x}$ ± S.E.) of male rats to i.a. infusions of NOR (4.30 μg) and ANG (1.25 μg) following 4 weeks of isolation stress. Groups are DUM, OL, and EPA in OL.
* p<0.05 vs. OL ** p<0.01 vs. OL *** p<0.001 vs. OL
† p<0.05 vs. DUM

the duration of response ($p<0.01$) and diastolic ($p<0.01$) responses to ANG vs OL. There were no differences in response to ANG between DUM and EPA groups.

DISCUSSION

In previous reports, 18:3ω6 and 20:4ω6, but not 18:2ω6 or 18:3ω3, attenuated pressor responses to chronic stress (5). However, it was not known whether the inability of 18:3ω3 to reduce BP resulted from its not being in the ω6 series, or rather because it is a pre- Δ6- and Δ5-desaturase compound (5). The results of the present study suggest that the ω3 series fatty acid, EPA (20:5ω3), a post-Δ6- and Δ5-desaturase product, is capable of inhibiting pressor responses to chronic stress. In light of the normally high activity of Δ6- and Δ5-desaturase in the rat (11), and the observation that EPA, but not equimolar amounts of 18:3ω3, depress cardiovascular responses to stress, these data further support the hypothesis that chronic stress may inhibit fatty acid metabolism.

In addition, the data suggest that EPA, 18:3ω6, and 20:4ω6 may vary in both their ability to attenuate stress responses, as well as their mechanism of action. In a previous report (5), 18:3ω6 completely prevented a BP response over a 4 wk stress period, where as equimolar amounts of 20:4ω6 were effective only for 2 wks (5). Presently, a similar dose of EPA only partially inhibited the BP response over a 4 wk period. Should the potency of the various compounds differ, a higher EPA dose might completely suppress the BP response to stress. Furthermore, in the present study, EPA administration resulted in a marked increase in the tachycardic response to stress vs controls, whereas in previous reports, 18:3ω6 and 20:4ω6 treatment were associated with a suppression of stress-induced tachycardia (5). It is possible that the rise in heart rate during the suppression of BP by EPA reflects a baroreflex response. This would suggest that EPA may be acting peripherally, rather than centrally, in this model, possibly via inducing peripheral vasodilation.

Finally, the effects of EPA on reactivity to NOR and ANG do not appear to be responsible for the BP effects observed in the study.

Thus, the results of the present study suggest that EPA may be useful in attenuating cardiovascular reactivity to stress, and may provide a useful approach to a dietary reduction of stress reactivity in animals and humans.

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