A resveratrol derivative modulates TRH and TRH-like peptide expression throughout the brain and peripheral tissues of male rats

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Abstract

Introduction: Resveratrol and related polyphenols have therapeutic effects ranging from treatment of depression, Alzheimer’s and Parkinson’s disease, obesity, diabetes, neurodegeneration and ageing. TRH and TRH-like peptides, with the structure pGlu-X-Pro-NH2, where ‘X’ can be any amino acid residue, have reproductive, caloric-restriction-like, anti-ageing, pancreatic-β cell-enhancing, cardiovascular and neuroprotective effects. We hypothesize that TRH and TRH-like peptides are mediators of the therapeutic actions of the resveratrol derivative pterostilbene (PT).

Methods: Sixteen young adult male Sprague–Dawley rats were divided into four groups. Control group remained on ad libitum chow and water for 10 days. Acute group received ad libitum chow and water for 9 days and then 0.9 g PT/250 g rat chow for 24 h. Chronic animals received PT in chow for 10 days. Withdrawal rats received PT chow for 8 days and then normal chow for 2 days. TRH and TRH-like peptide levels were measured in medulla oblongata (MED), frontal cortex (FCX), hypothalamus (HY), amygdala (AY), hippocampus (HC), piriform cortex (PIR), nucleus accumbens (NA), entorhinal cortex (ENT), striatum (STR), cerebellum (CBL), anterior cingulate (ACNG), posterior cingulate (PCNG), prostate (PR), liver (L), testis (T), heart (H), pancreas (PAN), adrenals (AD) and epididymis (EP).

Results: Significant changes in the levels of TRH and TRH-like peptides occurred throughout the brain and peripheral tissues in response to PT treatment.

Conclusion: The high responsiveness of PIR, CBL, HY, STR, PCNG, MED, FCX, NA, ACNG and AY in brain and EP and PR is consistent with TRH and TRH-like peptides participating in the therapeutic effects of PT.

KEYWORDS
cerebellum, epididymis, hypothalamus, piriform cortex, prostate
Resveratrol and related polyphenols such as pterostilbene (PT) have therapeutic effects ranging from treatment of depression and anxiety,1–5 stress,6–8 PTSD,9 epilepsy,10 Alzheimer’s and Parkinson’s disease,11–13 diabetes,13,14 obesity,15 cancer,16–18 traumatic brain injury,5 Huntington’s disease,19 hypertension,20 pain,5,21 neurodegeneration22–25 and ageing.19,26–30 Resveratrol upregulates mitochondria-located antioxidant enzymes and triggers mitochondrial biogenesis.21

TRH and TRH-like peptides, with the structure pGlu-X-Pro-NH2, where ‘X can be any amino acid residue, have antidepressant, anti-epileptic, analeptic, reproductive, caloric-restriction-like, anti-ageing, pancreatic-β cell-enhancing, cardiovascular and neuroprotective effects.32 The TRH/TRH-R1 receptor signalling pathway is an important mediator of brain–gut axis communication via the brain medulla.33

TRH and TRH-like peptides occur not only throughout the CNS but also peripheral tissues, with particularly high levels in rat and human prostate.32,34 Resveratrol decreases both serum TSH and hypothalamic TRH mRNA expression in sub-clinically hypothyroid rats.2

Resveratrol promotes expression of sirtuins (SIRTs).28,33 SIRTs are a family of NAD+-dependent enzymes that catalyse post-translational modifications of proteins. They regulate cellular functions and are associated with ageing and longevity. Dysregulation of SIRTs plays an important role in major diseases, including cancer and metabolic, cardiac and neurodegenerative diseases.35

Sulphated metabolites accumulate in the gut following oral ingestion of resveratrol where they promote the growth of beneficial bacteria such as Lactobacillus reuteri and up-regulate the expression of tight junction and mucin-related proteins.36 Perturbation of the gut–blood barrier, has a profound effect on the expression of reproductive and brain TRH and TRH-like peptides.34

The present studies investigate the potential of TRH and TRH-like peptides being downstream mediators of PT because: (1) this polyphenol (see Figure 1) readily crosses the blood–brain barrier resulting in increased bioavailability, clearance time and therapeutic potential compared to resveratro37,38 and (2) TRH and TRH-like peptides are important mediators of intracellular functions, which overlap those of PT and resveratrol, but are rapidly degraded by blood enzymes and cannot cross blood–tissue barriers.32

2 | EXPERIMENTAL PROCEDURES

2.1 | Animals

‘Young adult male Sprague-Dawley rats (n = 16, SPF, Envigo) were used for all experiments. These animals were group housed (2 animals per cage) on wood shavings with a red plastic tube for play and shelter. Standard Purina rodent chow #5001 and water were provided ad libitum during a standard one-week initial quarantine with 22 ± 2°C and 50 ± 10% relative humidity; lights on: 6 am to 6 pm.

Cages, water and bedding were changed every 3 days. All animals were weighed on the day of receipt and on the morning of each experiment. Initial body weights did not differ between experimental groups. Animals were randomized prior to the start of PT treatment. Research was approved by the VA Greater Los Angeles Healthcare System Animal Care and Use Committee (IACUC Protocol #030090-10) and conducted in compliance with the Animal Welfare Act and the federal statutes and regulations related to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and use of Laboratory Animals, Eighth Edition, NRC Publication, 2011. All efforts have been made to minimize the number of animals used and their suffering. Animal was handled for 10min per day for one month and then transferred from the Veterinary Medical Unit to the laboratory 12h before the start of experiments to minimize the stress of a novel environment.32 ‘The American Veterinary Medical Association has concluded that decapitation without prior sedation “is conditionally acceptable if performed correctly, and it should be used in research settings when its use is required by the experimental design and approved by the Institutional Animal Care and Use Committee”’.39 This study is reported in accordance with ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) (https://arriveguidelines.org).

‘Because of the 10- to 100-fold changes in TRH and TRH-like peptide levels in response to the estrus cycle, female rats were not included in the present study’.40

2.2 | Effect of acute, chronic and withdrawal treatment with PT in normal rat chow on levels of TRH and TRH-like peptides in rat brain and peripheral tissues

Sixteen young adult male Sprague-Dawley rats (7 weeks), body weight (mean ± SD) 203 ± 6 g, were divided into four groups (n = 4/group). PT chow was prepared by adding 0.9 g PT (Sigma) to 250 g of standard rodent chow and blending thoroughly with a Ninja Model BL610 Professional 1000W blender for 30s. The control (CON) group remained on ad libitum standard chow and water for 10 days until decapitation. The acute (AC) group received ad libitum chow and water for 9 days and then PT chow for 24h. Assuming 25 g chow consumption/day, this would provide 300mg PT/kg body weight for 300 g rats. The chronic (CHR) animals received PT chow for 10 days. The withdrawal (WD) rats received PT chow for 8 days and then normal chow for 2 days. The effect of PT withdrawal on TRH and TRH-like peptide levels when compared to the corresponding acute effects can reveal the relative contribution of changes in peptide biosynthesis (hours) to changes in peptide release (minutes).34

2.3 | Dissection of rat brain and peripheral tissues

All rats were decapitated without anaesthesia to avoid rapid, anaesthetic-induced, blockade of peptide release.41 Nucleus
accumbens (NA), amygdala (AV), frontal cortex (FCX), cerebellum (CBL), medulla oblongata (MED), anterior cingulate (ACNG), posterior cingulate (PCNG), striatum (STR), piriform cortex (PIR), hippocampus (HC), entorhinal cortex (ENT), adrenals (AD), pancreas (PAN), prostate (PR), epididymis (EP), tests (T), heart (H) and liver (L) were hand dissected, weighed rapidly and then extracted as previously described in detail.32

2.4 | Serum hormone assays

Serum rat leptin, rat insulin, testosterone, free T₄, total T₃ and glucose were measured (assay range, intra-assay CV%) with the following commercial RIA kits: rat leptin (0.801–200 ng/ml, 3.2) and rat insulin (0.0329–2.0 ng/ml, 4.8) (Linco Research, Inc.), testosterone (0.05–40 ng/ml, 6.7), free T₄ (0.045–60 ng/dl, 4.6) and total T₃ (0.06–80 pg/ml, 4.8) (MP Biomedical). Serum glucose was measured with the Contour Next EZ Blood Glucose Monitoring System (Ascensia Diabetes Care US, Inc.).

2.5 | HPLC and RIA procedures, HPLC peak identification and quantitation

HPLC and RIA procedures, peak identification, and quantitation by co-chromatography with synthetic TRH and TRH-like peptides, relative potency analysis of multiple antibodies to TRH and TRH-like peptides, and mass spectrometry for comparing peak areas have been previously reported in detail.32–45

Briefly, after boiling, tissues were dried, re-extracted with methanol, dried and defatted by water–ethyl ether partitioning. Dried samples were dissolved in 0.1% trifluoroacetic acid (TFA) and loaded onto reverse phase C18 Sep-Pak cartridges (Water). TRH and TRH-like peptides were eluted with 50% methanol. Dried peptides were again dissolved in TFA, filtered and then fractionated by HPLC using a 4.6 mm × 150 mm Econosphere, 3 mm C18 reverse phase column (Dr. Maisch GmbH) and a 0.2%/min gradient of acetonitrile. The 0.5 ml fractions collected were dried completely and reconstituted with 0.10 ml of 0.02% NaN₃ just before RIA.

The antiserum used (8B9) cross-reacts with TRH and nine TRH-like peptides with a relative potency of displacement ranging from 2.31 (Lys-TRH) to 0.288 (Ser-TRH) relative to Tyr-TRH [Table 2, Ref. 42]. Two of the regularly observed peaks (2a and 2b) consist of a mixture of unidentified TRH-like peptides. Of the eight observed peptides, three have so far been confirmed by mass spectrometry: TRH, Glu-TRH and Tyr-TRH.33 Tissue samples from the 4 rats within each treatment group were pooled prior to HPLC to provide the minimum amount of immunoreactivity needed for reliable RIA measurements.

The mean recovery of TRH and TRH-like peptide immunoreactivity from all tissues studied was 84 ± 15% (mean ± SD). The within-assay and between-assay coefficient of variation for measuring 333 pg/ml TRH was 4.8% and 16.9%, respectively. All HPLC fractions obtained from a given brain region or peripheral tissue were analysed in the same RIA. The minimum detectable dose for TRH was 5 pg/ml. The specific binding of [125]TRH (Bo/T) was 25%.

2.6 | Statistical analysis

Statistical methods for comparing peak areas were made with the aid of Statview (Abacus Concepts, Inc.), a statistical software package for the Macintosh computer. All multi-group comparisons were carried out by one-way analysis of variance using post hoc Scheffe contrast with the control group.

The mean within-group coefficient of variation (CV) (SD/mean, CV-within group) for each tissue and TRH/TRH-like peptide combination, across four photoperiod intervals, has been previously reported (circadian rhythm experiment) for untreated Sprague-Dawley male rats.46 Mean within-group CVs in brain ranged from 4.5% for TRH levels in AY to 43% for Phe-TRH in HY, and from 12% for Val-TRH in testis to 41% for Trp-TRH in EP for peripheral tissues. These CVs were then used to estimate the level of significance, by one-way ANOVA, of changes in the pooled mean values (see Ref. [47]) of TRH and TRH-like peptide levels following acute (AC), chronic (CHR) and withdrawal (WD) ingestion of PT. Pooling of at least 4 tissue extracts was required to provide sufficient signal-to-noise in the RIA for many brain regions and to keep the total number of HPLC fractions to be analysed reasonable: 4 treatment groups × 19 tissues × 100 HPLC fractions/tissue pool = 7600 RIA samples for the present study. Without pooling the total number of HPLC fractions would have been 4 × 7600 = 30,400.

3 | RESULTS

3.1 | Body weights

Mean body weights for all animals at the time of decapitation were 333.5 ± 20.0 g. Mean animal weights for each PT treatment group did not differ significantly with the untreated controls by one-way ANOVA.
3.2 | Serum hormone levels following oral PT

Serum glucose levels for the CHR group were significantly lower than the CON group (p < .05). All other serum hormone levels did not differ significantly between corresponding experimental groups by one-way ANOVA (Table 1).

3.3 | Overview of TRH and TRH-like peptide data

Our combined HPLC-RIA methodology can resolve 10 TRH and TRH-like peptides: Glu-TRH, Peaks 2a and 2b (partially resolved mixture of TRH-like peptides), TRH, Val-TRH, Thr-TRH, Tyr-TRH, Leu-TRH, Phe-TRH and Trp-TRH. The present study evaluated 12 brain regions and 7 peripheral tissues for the PT experiment. This represents 10 × 19 = 190 peptide mean values.

The number of significant changes in TRH and TRH-like peptide levels in brain resulting from PT treatment (in parentheses), in descending order were as follows: PIR(16), CBL(16), HY(15), STR(14), ACNG(12), MED(11), NA(10), AY(10), PCNG(10), PCX(7) as seen in Table 1 and Figures 2 and 3. The corresponding ranking for peripheral tissues were as follows: EP(17), PR(13), AD(8), H(5), T(4), L(3) and PAN(2) (See Table 3 and Figure 3).

4 | DISCUSSION

Acute, chronic and withdrawal treatment with PT results in significant increases in TRH, Leu-TRH, Trp-TRH, Phe-TRH, Tyr-TRH, Glu-TRH and Peak 2 and decreases in Val-TRH for piriform cortex (Table 2 and Figure 2). These changes result from alterations in the biosynthesis and/or release of these tripeptides. These remarkable changes in peptide levels within the piriform cortex are consistent with current knowledge regarding the role of TRH (and TRH-like peptides) as mediators of antidepressant effects in mammalian brain. The antidepressant activity of Tyr-TRH and analeptic effect of Val-TRH correspond with actions of TRH. TRH and TRH-like peptides have antidepressant effects. Resveratrol can reverse the dysregulation of limbic hypothalamus–pituitary–adrenal axis function and activation of neuroprotective molecules such as protein kinase A, phosphorylated cAMP response element-binding protein and brain-derived neurotrophic factor expression. Antidepressant-like effect of trans-resveratrol involves the regulation of the central serotonin and noradrenaline levels and related MAO-A activities.

Treatment with PT increased STR levels of TRH-like peptides including Tyr-TRH, Phe-TRH and Val-TRH (Table 2 and Figure 2) which have antidepressant effects. Resveratrol can reverse the dysregulation of mitochondrial respiration in models of Huntington’s disease which selectively affects the striatum and cortex.

Significant increases in TRH levels in posterior cingulate accompanied PT treatments (Table 2 and Figure 2). The posterior cingulate shows abnormalities in a range of neurological and psychiatric disorders including Alzheimer’s disease, schizophrenia, autism, depression, attention deficit hyperactivity disorder and ageing. Resveratrol treatment normalizes the peripubertal stress-induced social investigation deficit.

Microinjection of resveratrol into rostral ventrolateral medulla decreases sympathetic vasomotor tone through nitric oxide and intracellular Ca2+ in anaesthetized male rats. The resulting reduction in neuronal firing results in a decrease in heart rate and blood pressure. These effects are reversed by blockade of the nNOS pathway, suggesting that nitric oxide plays a role in mediating the sympathomimetic effects of resveratrol.

TABLE 1 Effect of oral pterostilbene on serum hormone levels of male rats

|          | Testosterone Nmol/L | fT3 pg/ml | fT4 ng/dl | Leptin ng/ml | Rat insulin ng/ml | Glucose mg/dl | CORT ng/ml |
|----------|---------------------|-----------|-----------|-------------|------------------|---------------|------------|
| CON      | 10.0 ± 4.2          | 2.61 ± 0.30| 2.49 ± 0.42| 1.40 ± 0.25  | 0.15 ± 0.04     | 97 ± 10       | 139 ± 32   |
| AC       | 11.5 ± 3.1          | 1.90 ± 0.35| 2.02 ± 0.17| 1.86 ± 0.51  | 0.15 ± 0.01     | 109 ± 11      | 301 ± 35   |
| CHR      | 10.1 ± 7.2          | 2.43 ± 0.45| 2.43 ± 0.43| 1.79 ± 0.27  | 0.15 ± 0.01     | 97 ± 6*       | 238 ± 35   |
| WD       | 12.3 ± 7.1          | 2.54 ± 0.46| 2.51 ± 0.49| 1.67 ± 0.16  | 0.15 ± 0.02     | 120 ± 5       | 243 ± 102  |

Note: All values are mean ± SD.

Abbreviations: AC, acute; CHR, chronic; CON, control; WD, withdrawal.

*p < .05 by one-way ANOVA using post hoc Scheffe contrasts with control rats.
| Glu-TRH | Peak 2 | TRH | Val-TRH | Tyr-TRH | Leu-TRH | Phe-TRH | Trp-TRH |
|---------|--------|-----|---------|---------|---------|---------|---------|
| **Hypothalamus** | | | | | | | |
| CON | 1641 ± 542 | 21,898 ± 7883 | 27,404 ± 8769 | 1727 ± 535 | 832 ± 216 | 1180 ± 484 | 435 ± 187 | 726 ± 203 |
| AC | 1423 ± 470 | 6307 ± 2271 | 59,590 ± 19,069 | 28,062 ± 8699** | 3063 ± 796* | 6751 ± 2768* | 2845 ± 1223* | 727 ± 204 |
| CHR | 333 ± 110** | 2339 ± 842** | 162,406 ± 51,970* | 1790 ± 555 | 1430 ± 372 | 1197 ± 491 | 703 ± 302 | 374 ± 105 |
| WD | 7952 ± 2624** | 19,431 ± 6995 | 50,078 ± 16,025 | 4246 ± 1316* | 1803 ± 469* | 30,851 ± 12,649*** | 4525 ± 1946** | 13,094 ± 3666** |
| **Amygdala** | | | | | | | | |
| CON | 1144 ± 324 | 2878 ± 386 | 11,732 ± 528 | 2592 ± 246 | 1364 ± 232 | 1836 ± 494 | 1634 ± 255 | 1686 ± 357 |
| AC | 1142 ± 323 | 3039 ± 407 | 19,854 ± 893 | 1185 ± 113* | 1831 ± 311 | 1822 ± 490 | 1373 ± 214 | 1305 ± 277 |
| CHR | 1825 ± 516 | 2688 ± 360 | 26,207 ± 1179* | 1067 ± 101* | 2087 ± 355 | 949 ± 255 | 1293 ± 202 | 3444 ± 730* |
| WD | 2461 ± 696* | 13,951 ± 1869** | 22,514 ± 1013 | 2002 ± 190 | 2091 ± 355 | 2158 ± 581 | 4679 ± 730* | 4406 ± 934* |
| **Piriform cortex** | | | | | | | | |
| CON | 515 ± 102 | 1556 ± 230 | 804 ± 125 | 2817 ± 397 | 248 ± 51 | 372 ± 81 | 769 ± 158 | 227 ± 72 |
| AC | 2872 ± 569** | 11,505 ± 1703** | 21,613 ± 3372*** | 3557 ± 502 | 0 | 2417 ± 529** | 2902 ± 595* | 633 ± 202* |
| CHR | 296 ± 59 | 1445 ± 214 | 2358 ± 368* | 119 ± 17*** | 203 ± 42 | 424 ± 93 | 745 ± 153 | 274 ± 87 |
| WD | 2300 ± 455* | 26,206 ± 3878** | 16,312 ± 2545*** | 1298 ± 183* | 645 ± 132* | 7213 ± 1580*** | 6571 ± 1347** | 3949 ± 1256** |
| **Nucleus accumbens** | | | | | | | | |
| CON | 544 ± 58 | 1423 ± 115 | 6711 ± 852 | 433 ± 55 | 887 ± 100 | 473 ± 134 | 577 ± 65 | 299 ± 57 |
| AC | 766 ± 81 | 2056 ± 167 | 3694 ± 469 | 3138 ± 399** | 525 ± 59 | 701 ± 198 | 601 ± 68 | 327 ± 62 |
| CHR | 534 ± 57 | 2924 ± 237* | 8858 ± 1125 | 1146 ± 146* | 388 ± 44* | 445 ± 126 | 558 ± 63 | 456 ± 87 |
| WD | 1609 ± 171* | 4616 ± 374* | 6000 ± 762 | 7002 ± 889*** | 3304 ± 373* | 764 ± 216 | 1978 ± 224* | 1208 ± 231** |
| **Striatum** | | | | | | | | |
| CON | 3122 ± 375 | 23,845 ± 3529 | 68,139 ± 6746 | 1309 ± 204 | 1310 ± 204 | 1350 ± 162 | 1029 ± 50 | 1007 ± 107 |
| AC | 5888 ± 707 | 17,100 ± 2531 | 38,066 ± 3769 | 4888 ± 763* | 4168 ± 650* | 3160 ± 379* | 2584 ± 127* | 3202 ± 339* |
| CHR | 4674 ± 561 | 7000 ± 1036* | 47,714 ± 4724 | 5398 ± 842* | 2518 ± 393 | 3486 ± 418* | 1516 ± 74 | 2480 ± 263* |
| WD | 3392 ± 407 | 64,982 ± 9617* | 35,520 ± 3516 | 7642 ± 1192* | 2168 ± 338 | 2168 ± 260 | 2520 ± 123* | 2716 ± 288* |
| **Medulla oblongata** | | | | | | | | |
| CON | 5730 ± 974 | 25,344 ± 2332 | 8537 ± 1024 | 2998 ± 402 | 3662 ± 725 | 2331 ± 611 | 1200 ± 246 | 1620 ± 309 |
| AC | 4631 ± 787 | 29,077 ± 2675 | 34,156 ± 4099** | 11,562 ± 1549* | 6002 ± 1188 | 3954 ± 1036 | 2923 ± 599* | 1831 ± 350 |
| CHR | 4878 ± 829 | 10,688 ± 983 | 11,391 ± 1367 | 35,069 ± 4699** | 3088 ± 611 | 5683 ± 1489* | 2347 ± 481 | 2812 ± 537 |
| WD | 9090 ± 1545 | 47,888 ± 4406 | 39,604 ± 4752** | 8240 ± 1104* | 10,220 ± 2024* | 5607 ± 1469* | 7344 ± 1506** | 4414 ± 843* |
| **Cerebellum** | | | | | | | | |
| CON | 1264 ± 99 | 3646 ± 259 | 10,038 ± 1345 | 4715 ± 500 | 2357 ± 332 | 5004 ± 1626 | 1670 ± 189 | 1276 ± 361 |
| (Continues) | | | | | | | |
| Glu-TRH | Peak 2 | TRH | Val-TRH | Tyr-TRH | Leu-TRH | Phe-TRH | Trp-TRH |
|---------|--------|-----|---------|---------|---------|---------|---------|
| AC 3309 ± 258 | 15.612 ± 1108** | 9059 ± 1214 | 9574 ± 1015* | 9355 ± 1319* | 2761 ± 897 | 4293 ± 485* | 2282 ± 646 |
| CHR 2807 ± 219* | 11.569 ± 821* | 10.478 ± 1404 | 11.449 ± 1214* | 2286 ± 322 | 4477 ± 1455 | 3465 ± 392* | 2034 ± 576 |
| WD 4945 ± 386* | 47.624 ± 3381** | 27.482 ± 3683* | 8040 ± 852 | 12.811 ± 1806** | 13.275 ± 4314* | 8475 ± 958** | 12.313 ± 3485*** |

**Anterior cingulate**

| CON | 997 ± 99 | 3268 ± 438 | 2494 ± 546 | 1001 ± 198 | 476 ± 74 | 1409 ± 478 | 896 ± 228 | 579 ± 115 |
| AC 959 ± 95 | 1538 ± 206* | 3512 ± 769 | 791 ± 157 | 441 ± 69 | 908 ± 308 | 1051 ± 268 | 507 ± 100 |
| CHR 225 ± 22** | 1404 ± 188* | 3203 ± 701 | 262 ± 52* | 524 ± 82 | 213 ± 72** | 461 ± 118 | 312 ± 62 |
| WD 1473 ± 146 | 3797 ± 509 | 11.383 ± 2493** | 319 ± 63* | 2074 ± 324** | 225 ± 76** | 915 ± 233 | 1778 ± 352* |

**Posterior cingulate**

| CON | 1664 ± 341 | 3796 ± 725 | 3671 ± 389 | 3267 ± 670 | 886 ± 144 | 3019 ± 1132 | 1924 ± 327 | 1538 ± 337 |
| AC 3527 ± 723* | 4552 ± 869 | 20.366 ± 2159** | 619 ± 127** | 2744 ± 447* | 1300 ± 488* | 1507 ± 256 | 1073 ± 235 |
| CHR 2280 ± 467 | 518 ± 99** | 41.896 ± 4441*** | 1990 ± 408 | 1175 ± 192 | 1795 ± 673 | 3011 ± 512 | 1427 ± 313 |
| WD 2743 ± 562 | 20.990 ± 4009** | 55.033 ± 5833*** | 36.165 ± 7414*** | 2041 ± 333* | 3017 ± 1131 | 5987 ± 1018* | 1950 ± 427 |

**Frontal cortex**

| CONa | — | — | — | — | — | — | — | — |
| AC 4055 ± 487 | 16.997 ± 4334 | 8691 ± 1721 | 6410 ± 949 | 1160 ± 74 | 3744 ± 528 | 6492 ± 824 | 1344 ± 176 |
| CHR 3325 ± 399 | 6155 ± 1569 | 34.929 ± 6916 | 2089 ± 309 | 1356 ± 87 | 1971 ± 278 | 4526 ± 575 | 2687 ± 352 |
| WD 11.012 ± 1321b | 34.751 ± 8862 | 100.922 ± 19983b | 120.801 ± 17.879c | 10.652 ± 682c | 50.476 ± 7117c | 484.100 ± 61.481c | 13.050 ± 1710c |

Note: aCON group for pooled FCX lost during extraction process. b<.01, c<.001 versus the AC group. All values are mean ± SD.

* p < .05; ** p < .01 and *** p < .002 by one-way ANOVA using post hoc Scheffe contrasts versus the CON group.

Abbreviations: AC, acute; CHR, chronic; CON, control; FCX, frontal cortex; WD, withdrawal.
FIGURE 2  Representative profiles of TRH and TRH-like peptide responses in male rats to pterostilbene (PT) treatment. The response profiles in (A–C) could be explained by PT-induced peptide biosynthesis along with a compensatory increase in peptide release. Withdrawal of PT results in a rapid decrease in peptide release (further increase in peptide level) while onset of decline in PT-induced biosynthesis is much slower. The profile in (D) suggests PT-induced peptide release, which is compensated by increased PT-dependent peptide biosynthesis. Upon PT withdrawal, synthesis declines but increased release persists. *p < 0.05; **p < 0.01; ***p < 0.002

FIGURE 3  Representative profiles of TRH and TRH-like peptide responses in male rats to pterostilbene (PT) treatments. The response patterns in (A–D) are consistent with PT-stimulation of rapid-onset but a slowly deceasing rate of PT-dependent biosynthesis which is compensated by a slow-onset increase in peptide release. Upon PT withdrawal, peptide release stops abruptly (increased peptide level) while PT-induced biosynthesis declines much more slowly. *p < 0.05; **p < 0.01; ***p < 0.002
| TABLE 3 | Effect of oral pterostilbene on TRH and TRH-like peptide levels in peripheral tissues of male rats (pg) |
|---------|--------------------------------------------------------------------------------------------------|
|         | Glu-TRH- | Peak 2 | TRH | Val-TRH | Tyr-TRH | Leu-TRH | Phe-TRH | Trp-TRH |
| Adrenals |          |        |     |         |         |         |         |         |
| CON     | 1202 ± 385 | 4739 ± 711 | 1951 ± 390 | 1214 ± 352 | 2098 ± 965 | 2233 ± 737 | 869 ± 235 | 949 ± 332 |
| AC      | 1033 ± 331 | 4779 ± 717 | 2854 ± 571 | 626 ± 182 | 816 ± 375° | 1737 ± 573 | 2972 ± 802° | 1286 ± 437 |
| CHR     | 785 ± 251 | 1872 ± 281° | 809 ± 162° | 1457 ± 423 | 712 ± 328° | 1016 ± 335° | 943 ± 255 | 1621 ± 551 |
| WD      | 1223 ± 391 | 5185 ± 778 | 3779 ± 756 | 1907 ± 553 | 4210 ± 1937° | 3996 ± 1319 | 2876 ± 777° | 119 ± 380 |
| Epididymis |          |        |     |         |         |         |         |         |
| CON     | 10,138 ± 3143 | 5175 ± 1708 | 5736 ± 1434 | 4389 ± 1448 | 14,166 ± 4385 | 6655 ± 1464 | 14,284 ± 1714 | 4537 ± 1860 |
| AC      | 24,905 ± 7721° | 37,767 ± 12,463° | 13,296 ± 332° | 2749 ± 907 | 2425 ± 728°** | 11,414 ± 2511 | 25,422 ± 3051 | 27,850 ± 11,419° |
| CHR     | 10,764 ± 3337 | 8645 ± 2853 | 2804 ± 701° | 4157 ± 1372 | 1500 ± 450°** | 2232 ± 491° | 1955 ± 235°** | 1702 ± 698° |
| WD      | 43,488 ± 13,481°** | 116,684 ± 38,506°*** | 56,272 ± 14,068°** | 39,955 ± 13,185°** | 19,395 ± 5819 | 90,957 ± 20,011°** | 49,501 ± 5940°* | 54,245 ± 22,240°** |
| Prostate |          |        |     |         |         |         |         |         |
| CON     | 211 ± 72 | 8059 ± 2579 | 259,466 ± 70,056 | 20,572 ± 5554 | 87,170 ± 27,023 | 195,010 ± 72,154 | 60,608 ± 18,788 | 72,909 ± 22,602 |
| AC      | 2018 ± 686°** | 24,966 ± 7989°* | 692,873 ± 18,7076°* | 91,780 ± 24,781°* | 210,706 ± 65,319°* | 113,261 ± 41,907°* | 89,028 ± 27,599 | 56,383 ± 17,479 |
| CHR     | 1244 ± 423° | 63,305 ± 20,258° | 94,085 ± 25,403° | 15,422 ± 4164 | 35,845 ± 11,112° | 91,463 ± 33,841° | 38,344 ± 11,887 | 18,164 ± 5631 |
| WD      | 12,379 ± 4209°*** | 40,485 ± 12,955° | 461,850 ± 124,700 | 81,411 ± 21,981° | 130,464 ± 40,444 | 165,815 ± 61,352 | 82,344 ± 25,527 | 72,961 ± 22,618 |
| Testis  |          |        |     |         |         |         |         |         |
| CON     | 572 ± 132 | 2621 ± 996 | 3131 ± 658 | 4745 ± 569 | 837 ± 243 | 2716 ± 760 | 2333 ± 537 | 383 ± 115 |
| AC      | 1038 ± 239 | 5040 ± 1915 | 4819 ± 1012 | 11,720 ± 1406° | 513 ± 149 | 4945 ± 1385 | 3960 ± 911 | 846 ± 254° |
| CHR     | 541 ± 124 | 2750 ± 1045 | 2200 ± 462 | 4405 ± 529 | 2480 ± 719° | 1521 ± 426 | 2055 ± 473 | 455 ± 137 |
| WD      | 288 ± 66° | 2909 ± 1109 | 1753 ± 368 | 5292 ± 635 | 1271 ± 369 | 4114 ± 1152 | 3206 ± 737 | 413 ± 124 |
| Pancreas |          |        |     |         |         |         |         |         |
| CON     | 1265 ± 443 | 3450 ± 1173 | 2312 ± 971 | 834 ± 192 | 1174 ± 247 | 1551 ± 357 | 819 ± 172 | 1641 ± 361 |
| AC      | 945 ± 331 | 2873 ± 977 | 1890 ± 794 | 1468 ± 338 | 757 ± 159 | 2203 ± 507 | 964 ± 203 | 2078 ± 457 |
| CHR     | 2117 ± 741 | 6234 ± 2120 | 2503 ± 1051 | 2367 ± 544° | 1136 ± 239 | 1622 ± 373 | 904 ± 190 | 1690 ± 372 |
| WD      | 2097 ± 734 | 2688 ± 914 | 1777 ± 746 | 1335 ± 307 | 2087 ± 438° | 1387 ± 319 | 1471 ± 309 | 688 ± 151 |
| Liver   |          |        |     |         |         |         |         |         |
| CON     | 1655 ± 397 | 3660 ± 695 | 3207 ± 1251 | 828 ± 240 | 1415 ± 425 | 674 ± 216 | 883 ± 283 | 1267 ± 291 |
| AC      | 1252 ± 300 | 3181 ± 604 | 1064 ± 415° | 1021 ± 296 | 601 ± 180 | 871 ± 279 | 1237 ± 396 | 1321 ± 304 |
| CHR     | 1325 ± 318 | 2741 ± 521 | 789 ± 308° | 769 ± 223 | 655 ± 197 | 1531 ± 490 | 3732 ± 1194° | 664 ± 153 |
| WD      | 1466 ± 352 | 4111 ± 781 | 1367 ± 533 | 861 ± 250 | 1995 ± 599 | 1081 ± 346 | 1473 ± 471 | 1764 ± 406 |
| Heart   |          |        |     |         |         |         |         |         |
| CON     | 232 ± 49 | 953 ± 123 | 1945 ± 175 | 1000 ± 220 | 1120 ± 246 | 1592 ± 350 | 2999 ± 720 | 610 ± 153 |
in blood pressure, heart rate and renal sympathetic nerve activity is consistent with a decreased TRH release rate resulting in increased TRH levels, Table 2, following acute and withdrawal treatment with PT. TRH increases blood pressure, heart rate and renal sympathetic nerve activity.32

Chronic social defeat stress, a model of depression in rodents, increases SIRT1 levels in the nucleus accumbens, a key brain reward region. Resveratrol, a pharmacological activator of SIRT1, when infused bilaterally into the NA, increased depression- and anxiety-like behaviours.56 Increased levels of TRH in NA following acute, chronic and withdrawal PT treatment (Table 2), is consistent with increased biosynthesis and release of TRH which has antidepressant and anxiolytic actions in male rats.32 Anterior cingulate inputs to the nucleus accumbens control the social transfer of pain and analgesia.57 The marked PT-induced changes in TRH and TRH-like peptide levels (Table 2, Figures 2 and 3) within the cingulate and nucleus accumbens are noteworthy given these important neural and cognitive linkages between these brain regions. SIRT1 in the brain is involved with ageing-associated disorders and lifespan.58

The posteromedial nucleus of the cortical amygdala contains TRH-expressing neurons that control mating behaviour.59 Chronic PT treatment increased TRH levels in the amygdala. (Table 2). Amygdala TRH levels fluctuate significantly during the rat oestrus cycle.40

High levels of transcriptional activity occur within the epididymis.32 Resveratrol improves sperm DNA quality and reproductive capacity in type 1 diabetes.60 The highest levels of Glu-TRH, which is a sperm capacitation factor,32 occur within the epididymis and were significantly increased by acute and withdrawal treatments with PT (Table 3).

The tissue in male rats with the highest levels of TRH and TRH-like peptides is the prostate. The TRH levels are subject to a 12-fold variation during the 24-h photoperiod with highest level during the diurnal period.32 Because rats are nocturnal while humans are most active during the day, this may explain the approximately 10-fold higher levels of rat TRH immunoreactivity (TRH-IR) in daytime compared to humans.32 The highly significant increases in Glu-TRH levels in response to PT treatments (Table 3 and Figure 3) are of particular interest because prostate cancer in particular, and other cancers, in general, have been found to be associated with nerves18 which are the main source of these peptides. They are co-secreted with glutamate and other neurotoxic stress-related neurotransmitters.32 Prostatic fluid contains TRH and other TRH-like peptides and appears to be secreted by epithelial cells.32

Oral PT has been reported to improve stress-related behaviours, neuroinflammation and hormonal changes in a mouse stress model.6 Acute and chronic PT treatment decreased adrenal TRH, Tyr-TRH, Leu-TRH and Phe-TRH levels in the adrenals (Table 3) consistent with increased release of these antidepressant peptides.32,44

Chronic treatment with PT reduced serum glucose (Table 1) and increased pancreatic Val-TRH and decreased Tyr-TRH levels
5 | CONCLUSIONS

Acute, chronic and withdrawal treatment with oral PT has significant effects on the expression of TRH and TRH-like peptides throughout the brain and peripheral tissues of male rats. These effects are consistent with these tripeptides playing a significant role in the antidepressant, anti-ataxic, anti-autistic, neuroprotective, anti-hyperpertensive, anti-ageing, anxiolytic and reproductive effects of this resveratrol analog which readily crosses the blood–brain barrier and thereby enhances its bioavailability.

AUTHOR CONTRIBUTIONS

Albert Eugene Pekary: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). Albert Sattin: Investigation (equal); methodology (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

All statistically summarized data are included in this published article. Primary data are available from AEP upon reasonable request.

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