Chem. Senses 25: 173–180, 2000

Suppression of *S*-methylglutathione-induced Tentacle Ball Formation by Peptides and Nullification of the Suppression by TGF-β in *Hydra*

Yasuko Manabe, Hanae Yamazaki, Chiaki Fukuda, Kazuo Inoue, Tohru Fushiki and Kazumitsu Hanai[1]

Laboratory of Nutrition Chemistry, Division of Applied Life Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502 and [1]Laboratory of Physics, Kyoto Prefectural University of Medicine, Kyoto 603-8334, Japan

Correspondence to be sent to: Kazumitsu Hanai, Biosensing Laboratory, Department of Physics, Kyoto Prefectural University of Medicine, Nishitakatsukasa-cho 13, Taishogun, Kita-ku, Kyoto 603-8334, Japan. e-mail: hanai@koto.kpu-m.ac.jp

Abstract

Tentacle ball formation (TBF) in *Hydra* elicited by *S*-methylglutathione (GSM) was modulated by a number of biologically active peptides. *Hydra* fed on *Artemia*, which had been hatched in a common salt solution supplemented with LiCl and $ZnCl_2$, easily induced TBF in response to GSM after pretreatment with trypsin. After *Hydra* were treated with 100 pg/ml trypsin for 10 min, the response to GSM (TBF) was sensitively suppressed by acidic fibroblast growth factor and other biologically active peptides for >10 h. Various peptides, but not transforming growth factor beta (TGF-β), suppressed GSM-induced TBF in a specific pattern for each peptide. However, TGF-β was unique in that it did not suppress the response to GSM, but nullified the suppressive effect of other peptides. Only active TGF-β nullified the suppressive effect of the peptides, and the latent form of TGF-β neither suppressed GSM-induced TBF nor nullified the suppressive effect of other peptides. Members of the TGF-β family suppressed GSM-induced TBF. These results indicate that all peptides examined, except for TGF-β, suppressed the response to GSM in a manner specific to each peptide. This assay system would be useful in identification of biologically active peptides.

Introduction

S-Methylglutathione (GSM), a stimulant as potent as reduced glutathione, elicited the tentacle ball formation (TBF) response to GSM, is a component of the feeding behavior of *Hydra* and the response is modified by the presence of various peptides. Of all the components of *Hydra* feeding behaviors, only TBF was modified by the presence of various peptides. Interestingly, TBF is sensitively modulated by various biologically active peptides including platelet-derived growth factor (PDGF) (Hanai *et al.*, 1987) and acidic fibroblast growth factor (aFGF) (Hanai *et al.*, 1989). Modulation by the peptides (mainly suppression) was observed in the presence of GSM at concentrations specific to each peptide. Recently we found that trypsin treatment of live *Hydra* is required for GSM-induced TBF (Hanai and Matsuoka, 1995). It is likely that a subtle change in culture conditions of *Hydra* affects the behavior elicited by GSM, since GSM induces so many different but mutually related behavioral components in *Hydra*.

In this study, we report the culture conditions under which TBF is induced efficiently in *Hydra* by GSM, and also the modulation of the response to GSM by aFGF and other synthetic peptides. Further, we describe a novel effect of TGF-β, which specifically nullifies the suppressive effect of active peptides on GSM-induced TBF. We also examined in detail the effects of TGF-β and closely related peptides on GSM-induced TBF.

Materials and methods

Hydra culture

Hydra japonica were cultured as previously described (Hanai, 1998). Every 2 days, they were fed *Artemia naupli* (Argentemia Gold, Argent, Redmond, WA), which had been hatched in a solution of 30 g/l common salt (>99% NaCl, Japan Tobacco, Tokyo, Japan) containing 1 ng/l $ZnCl_2$ and 0.3 g/l LiCl (except for the experiment to examine the effect of salt composition), with aeration for 1 day at 28–30°C. A portion of *Hydra* from the mass culture was transferred to a new glass tray, and cultured for another 4 days before the behavioral test. The *Hydra* were fed on the first and the third day with *A. naupli* which had been hatched in a solution of 30 g/l common salt supplemented with 4.0 g/l $MgCl_2 \cdot 6H_2O$ with aeration for 1 day at 28–30°C. TBF of *Hydra* that were fed on *Artemia* hatched in the medium

supplemented with $MgCl_2$ just before experimental use was easily modulated by peptides.

Effect of salt composition of *Artemia* hatching solution

Artemia were hatched in a solution containing 30 g/l common salt (Japan Tobacco), 0.3 g/l LiCl and 10 ng/l of one of the following metal salts: $NaVO_3$, $MnCl_2{\cdot}4H_2O$, $FeCl_3{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $CuSO_4{\cdot}5H_2O$, $ZnCl_2$, $NaSeO_3$ and Na_2MoO_4. The effects of various concentrations of $ZnCl_2$ were examined in the presence or absence of 0.3 g/l LiCl. TBF of *Hydra* fed on *Artemia*, which had been grown in solutions containing each salt, was examined in the presence of 0.1 or 10 μM GSM for 10 h, after a 10 min pretreatment with trypsin (crystallized, porcine pancreas, 100 pg/ml; Wako Pure Chemical Co. Ltd, Osaka, Japan). In this experiment, *Hydra* in mass culture were fed on *Artemia* that had been hatched in the common salt solution without any supplements.

Trypsin treatment

Hydra were treated with 10, 100 or 1000 pg/ml trypsin for 10 min in 1 mM HEPES buffer containing 1 mM $NaHCO_3$, and 1 mM $CaCl_2$ (pH 7.7)—HEPES-buffered BC (Hanai and Matsuoka, 1995). After the designated period, ten *Hydra* individuals were transferred to a dish (35 mm in diameter) containing 2 ml PIPES solution (1 mM PIPES, 1 mM $CaCl_2$, pH 6.2), and the response to 10 μM GSM was examined in the presence or absence of 1 pg aFGF (Toyobo, Osaka, Japan).

Induction of TBF by GSM

After a brief rinse, ten *Hydra* individuals were transferred into a dish containing 2 ml of PIPES (1 mM PIPES, 1 mM $CaCl_2$, pH 6.2) and 1 μl of the test peptide in 0.2% Prionex (Merck, Darmstadt, Germany). The dish was placed on the stage of a binocular microscope, which was kept at 20°C by circulating temperature-regulated water. After 5 min, a small amount (<10 μl) of concentrated GSM solution was applied to make final concentrations of 0.1, 0.3, 3, 10 and 50 μM. At these concentrations of GSM, we observed the largest suppression of GSM-induced TBF by various biologically active peptides (Hanai *et al.*, 1987). After a gentle swirl, the number of *Hydra* that showed TBF was counted every minute for 10 min under a binocular microscope (×8). The response to GSM (TBF) was shown by the sum of the numbers of *Hydra* with TBF counted at each minute from 6 to 10 min divided by the total number of *Hydra* (Hanai and Matsuoka, 1995).

Sampling of rat cerebrospinal fluid (CSF)

Eight-week-old male Sprague–Dawley rats (Japan Charles River, Yokohama, Japan) were kept at 22 ± 2°C and 60% relative humidity under a 12 h:12 h light:dark cycle. The rats had free access to food and water. The rats were starved overnight before the day of the experiment and were anesthetized with pentobarbital. CSF was then collected from the cistema magna.

All animals received humane care as outlined in the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee, according to NIH No. 86-23, revised 1985).

Biologically active peptides used as modulators of GSM-induced TBF

The peptides tested were: aFGF and basic fibroblast growth factor (bFGF); PDGF (Becton Dickinson Laboware, Bedford, MA); calcitonin gene-related peptide (CGRP, human); cholecystokinin tetrapeptide (CCK4, 30–33); cholecystokinin octapeptide (CCK8, 26–33, non-sulfated form); corticotropin releasing factor (CRF, human); growth hormone releasing factor (GRF, human); neuropeptide Y (NPY, human); and substance P (human). All synthetic peptides were the products of Peptide Institute Inc. (Osaka, Japan).

Effect of TGF-β on GSM-induced TBF

CRF was used to suppress GSM-induced TBF because it is easily available and shows a stable suppressive effect at the highest GSM concentration (50 μM). The mixtures of CRF (1 ng) and TGF-β1, -β2 and -β3 (1 ng) were added to *Hydra* assay medium (2 ml) containing 50 μM GSM and TBF was observed.

Effect of the members of TGF-β superfamily on GSM-induced TBF

Latent TGF-β1 (1 ng, recombinant human latent TGF-β1, R&D Systems MN, USA) or active TGF-β1 (1 ng) was added to the *Hydra* assay medium (2 ml) containing GSM at various concentrations and TBF was observed. The effect of TGF-β1 on TBF in the presence of both GSM and 1 μl of rat CSF (diluted to 10^4 with 0.2% Prionex solution) was also examined. Rat CSF, which contains various biologically active peptides, strongly suppressed TBF induced by GSM at each concentration, as observed previously (Hanai *et al.*, 1989; Inoue *et al.*, 1999). The effect of the mixture of latent or active TGF-β1 and diluted rat CSF was also examined.

Effect of the members of TGF-β superfamily on GSM-induced TBF

We examined the effects of TGF-β1, BMP4 (recombinant, *Xenopus laevis*, a kind gift from Dr Naoto Ueno) (Nishimatsu *et al.*, 1992), human glial-derived neurotrophic factor (GDNF, Alomone Labs Ltd, Jerusalem, Israel), inhibin (human follicular fluid, Biogenesis Ltd, Poole, UK) and activin A (recombinant bovine activin A, Innogenetics NV, Zwijndrecht, Belgium) on GSM-induced TBF. The stock solutions of these peptides were prepared according to the manufacturers' directions and added after dilution with 0.2% Prionex. Peptides (1 ng) were added to the *Hydra* assay

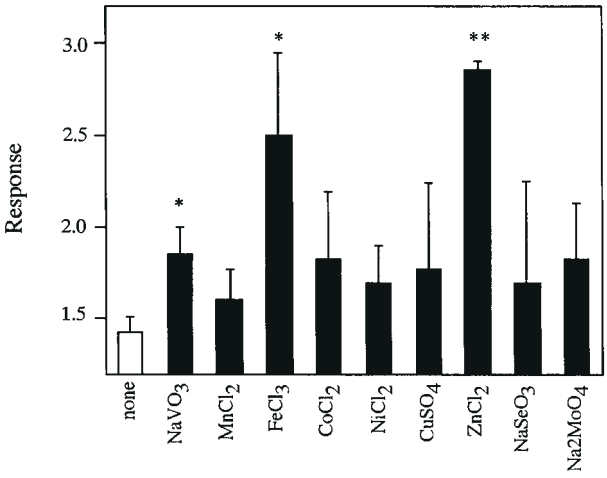

Figure 1 Effect of various metal ions added to the solution in which *Artemia* were hatched on GSM-induced TBF in *Hydra*. Metal compounds at 10 ng/l were added to the solution (30 g/l common salt and 0.3 g/l LiCl). *Hydra* was fed *Artemia* twice every other day before tests. None: *Artemia* were hatched in common salt solution (30 g/l) without any supplement. TBF was induced by 0.1 μM GSM. Response (ordinate) is the response to GSM (TBF). Values are means ± SD (n = 3–7). Statistically significant from none by Dunnet's test (*$P < 0.05$, **$P < 0.01$).

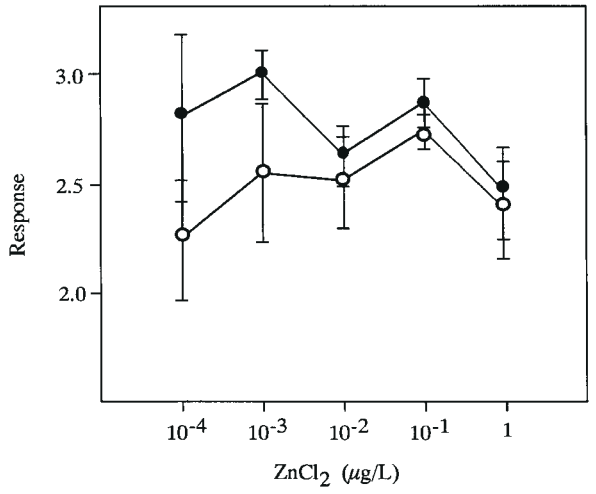

Figure 2 Effect of $ZnCl_2$ and LiCl added to the solution in which *Artemia* were hatched on GSM-induced TBF. $ZnCl_2$ at various concentrations was added together with 0.3 g/l LiCl (solid circle) or in the absence of LiCl (open circle). TBF was induced by 10 μM GSM. Values are means ± SD (n = 6–8). The response to GSM in the presence of LiCl is significantly different from that in the absence of LiCl ($P < 0.05$, ANOVA, difference of any pair at the same $ZnCl_2$ concentration was not significant by multiple comparisons).

medium (2 ml) containing GSM at various concentrations, and TBF was observed.

Statistics

All statistical tests were carried out using StatView (SAS Institute Inc., Cary, NC).

Table 1 Development of factor-sensitivity (TBF) of the response to GSM in *Hydra* potentiated by trypsin

	+aFGF		Control		Significance
Time (h)	Average ± SD	n	Average ± SD	n	
Potentiated by 10 pg/ml trypsin					
2	3.52 ± 0.77	5	3.58 ± 0.17	4	
4	3.30 ± 0.48	5	3.60 ± 0.52	4	
6	1.62 ± 0.63	5	3.35 ± 0.37	4	$P < 0.05$
8	1.38 ± 0.51	5	3.23 ± 0.24	4	$P < 0.01$
Potentiated by 100 pg/ml trypsin					
0.5	2.80 ± 0.41	4	3.58 ± 0.17	4	$P < 0.05$
1	1.86 ± 0.21	5	3.88 ± 0.25	4	$P < 0.01$
2	1.86 ± 0.67	5	3.48 ± 0.25	4	$P < 0.05$
5	1.84 ± 0.40	5	3.55 ± 0.21	4	$P < 0.05$
8	1.60 ± 0.33	5	3.30 ± 0.08	4	$P < 0.01$
Potentiated by 1 ng/ml trypsin					
0.5	2.68 ± 0.64	5	3.63 ± 0.32	3	
1	1.98 ± 0.37	5	3.53 ± 0.48	4	$P < 0.01$
3	2.38 ± 1.00	5	3.30 ± 0.24	4	
5	3.26 ± 0.67	5	3.48 ± 0.49	4	
8	3.42 ± 0.51	5	3.70 ± 0.32	4	

Statistical analysis was by paired t-test (two-tailed). Animals were treated with trypsin at specified concentrations for 5 min in BC solution, and then kept in BC solution without trypsin for a designated time (h). TBF was induced by 10 μM GSM in the presence or absence (control) of 0.1 pg/ml aFGF (+aFGF).

Results

Effect of metal ions in *Artemia* hatching solution on GSM-induced TBF

In preliminary experiments we found that the salt composition of the solution in which *Artemia* had been hatched affected GSM-induced TBF in *Hydra*. Since the common salt from Japan Tobacco was better for induction than the reagent grade NaCl, we assumed that minor heat-stable components contained in the former salt had a positive effect on GSM-induced TBF. We examined metal salts that are contained in the biological tissues as cofactors of transport proteins and enzymes.

Various salts were added to the *Artemia* hatching solution. Multivalent cations such as Fe^{3+} and Zn^{2+} effectively promoted TBF in response to 0.1 μM GSM (Figure 1). A similar effect of these ions was observed in the response to higher concentrations of GSM, although the effect was less apparent (data not shown). The effect of $ZnCl_2$ was stronger in the presence of LiCl, especially at lower concentrations of $ZnCl_2$ (Figure 2). After extensive examinations, 1 ng/l $ZnCl_2$ and 0.3 g/l LiCl were added to the common salt. In *Hydra* fed on *Artemia* hatched in the solution containing $FeCl_3$,

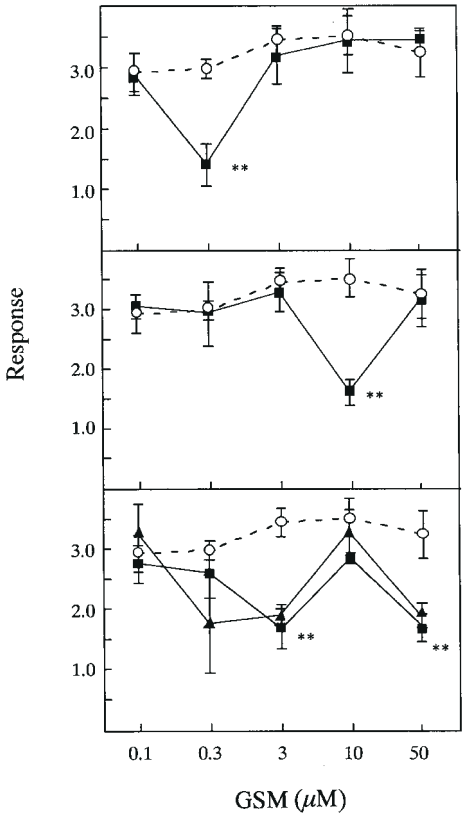

Figure 3 Effect of growth factors on TBF induced by GSM at various concentrations in *Hydra* potentiated by 100 pg/ml trypsin (10 min). GSM was given in the presence of 0.5 ng/ml PDGF (top), 0.5 pg/ml aFGF (middle) and 0.05 pg/ml or 0.5 ng/ml bFGF (bottom). Open circles on all figures are the control response values (the response in the absence of growth factors). Values are means ± SD ($n = 4$). Statistically significant from the control response by Student's *t*-test (**$P < 0.01$).

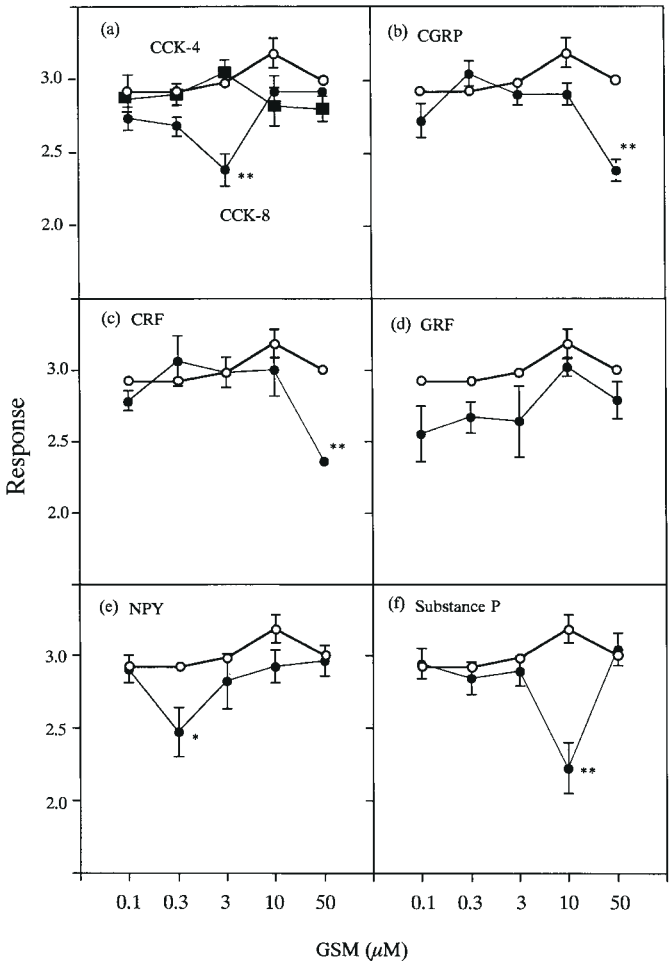

Figure 4 Effect of various synthetic peptides on TBF induced by GSM at various concentrations. **(a)** CCK4 (solid square); CCK8 (solid circle). **(b)** CGRP (solid circle). **(c)** CRF (solid circle). **(d)** GRF (solid circle). **(e)** NPY (solid circle). **(f)** Substance P (solid circle). The concentration of each peptide was 0.5 ng/ml. Open circles are values of control response, which were examined in the absence of peptide. Values are means ± SD ($n = 5$). Statistically significant from the control response by Student's *t*-test (*$P < 0.05$, **$P < 0.01$).

GSM-induced TBF tended to be less modulated by peptides (unpublished observation).

Modulation of GSM-induced TBF by peptides

When *Hydra* were treated with trypsin at concentrations <1 μg/ml for 10 min, they developed a new repertory of GSM-induced behavior, TBF, as reported earlier (Hanai and Matsuoka, 1995; Hanai, 1998). When the concentration of trypsin was 100 pg/ml, TBF was suppressed by aFGF for >8 h (Table 1). When the trypsin concentration was 10 pg/ml, the suppression was observed a few hours later. On the other hand, when the trypsin concentration was 1 ng/ml, the suppression was observed during the first few hours, but not thereafter.

We examined TBF induced by GSM at various concentrations in the presence of PDGF, aFGF or bFGF (Figure 3). In this experiment, *Hydra* were pretreated with 100 pg/ml trypsin for 10 min. Only TBF induced by GSM at the concentration specific for each factor was suppressed by that factor, as reported earlier, when *Hydra* were not pretreated with trypsin. In our previous experiments, trypsin was not required to produce GSM-induced TBF for reasons which are unclear (Hanai *et al.*, 1987, 1989). The above factors seem to suppress GSM-induced TBF similarly, either with or without pretreatment with trypsin.

We also examined the effect of some synthetic peptides on GSM-induced TBF. Figure 4 shows the effects of cholecystokinin peptides (CCK), CGRP, CRF, GRF, NPY and substance P. The effects of these peptides were examined at a concentration of 0.5 ng/ml. The minimum concentration of CRF required to suppress TBF was 0.5 fg/ml. GSM-induced TBF was suppressed by the synthetic peptides only when the GSM concentration was at the range specific to the peptide (Figure 4).

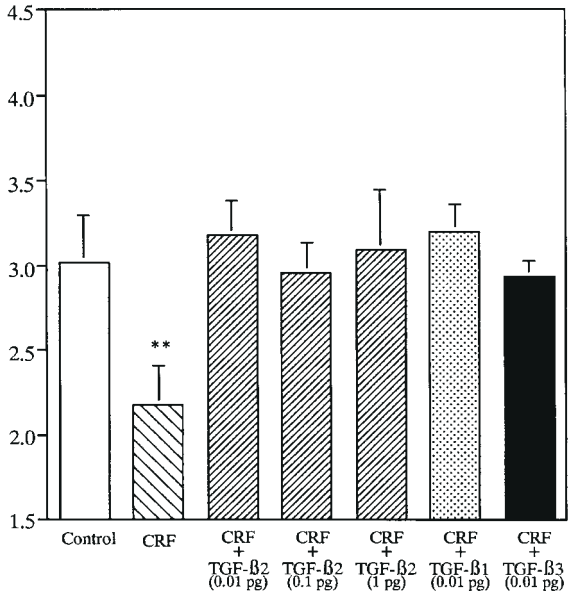

Figure 5 Nullification of the suppressive effect of CRF on TBF, induced by 50 μM GSM, by isoforms of TGF-β. Control response was observed in the diluent, 0.2% Prionex (1 μl). Values are means ± SD ($n = 5$). **Significantly different from the control by Dunnet's test ($P < 0.01$).

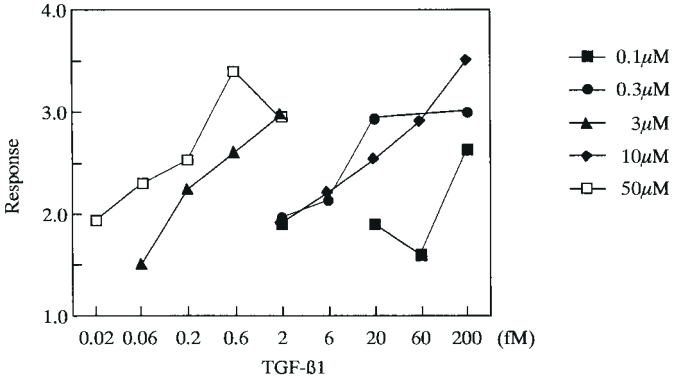

Figure 6 Concentration-dependent effect of TGF-β1 on TBF suppressed by CSF (1 μl of the 10^4 diluted CSF) in the presence of GSM at various concentrations.

Effect of TGF-β on GSM-induced TBF

CRF suppressed TBF induced by 50 μM GSM (Figure 5). However, when TGF-β2 was added to the medium together with CRF, the suppressive effect of CRF was nullified. Three distinct forms of TGF-β have been identified in mammals (TGF-β1, -β2 and -β3) (Massague, 1990), and any one of them nullified the suppressive effect of CRF similarly. No difference in the suppression-nullifying effects was observed among the three TGF-β isoforms (Figure 5).

We examined the effect of TGF-β1 on GSM-induced TBF that was suppressed by CSF. CSF is known to suppress TBF induced by GSM (Hanai *et al.*, 1989; Inoue *et al.*, 1999). Different concentrations of TGF-β1 were required to nullify the suppressive effect of CSF depending on the concentration of GSM used to induce TBF (Figure 6). The concentration of TGF-β1 required to nullify the suppressive effect of CSF was >200, >20, >0.2, >20 and >0.06 fM in the presence of 0.1, 0.3, 3, 10 and 50 μM GSM, respectively. The suppressive effect of CSF was the most sensitive to TGF-β1 when TBF was induced by 50 μM GSM.

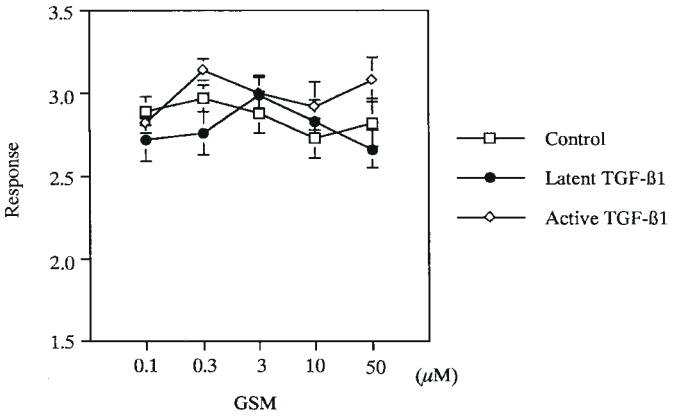

Figure 7 Effect of latent and active TGF-β1 (1 ng) on TBF induced by GSM at various concentrations. Control response was observed in the presence of the diluent, 0.2% Prionex (1 F128ml). Neither form of TGF-β1 suppressed the response to GSM. Values are means ± SEM ($n = 6$–8).

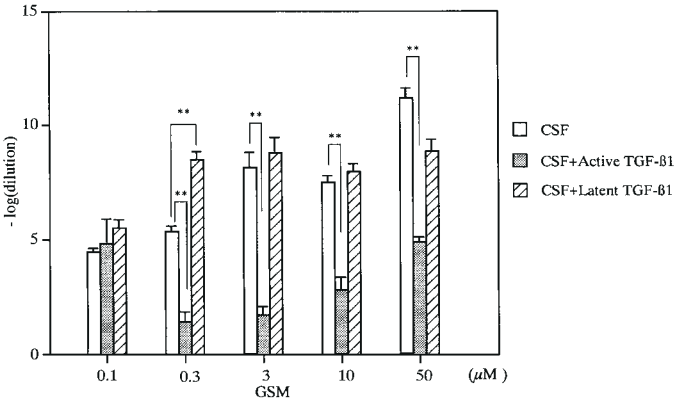

Figure 8 Suppressive activity of CSF, CSF + active TGF-β1 (10 pg) and CSF + latent TGF (1 ng) on GSM-induced TBF. The suppressive activity was expressed as the maximum dilution at which the suppression of GSM-induced TBF was observed. Values are means ± SEM (CSF, $n = 9$; active TGF-β1, $n = 6$; latent TGF-β1, $n = 5$). **Significantly different from values with CSF by Dunnet's test ($P < 0.01$).

Effect of active and latent TGF-β1 on GSM-induced TBF

Neither latent nor active TGF-β1 suppressed GSM-induced TBF (Figure 7). Active TGF-β1 greatly reduced the suppressive effect of rat CSF on GSM-induced TBF (Figure 8). However, latent TGF-β1 did not reduce the suppressive

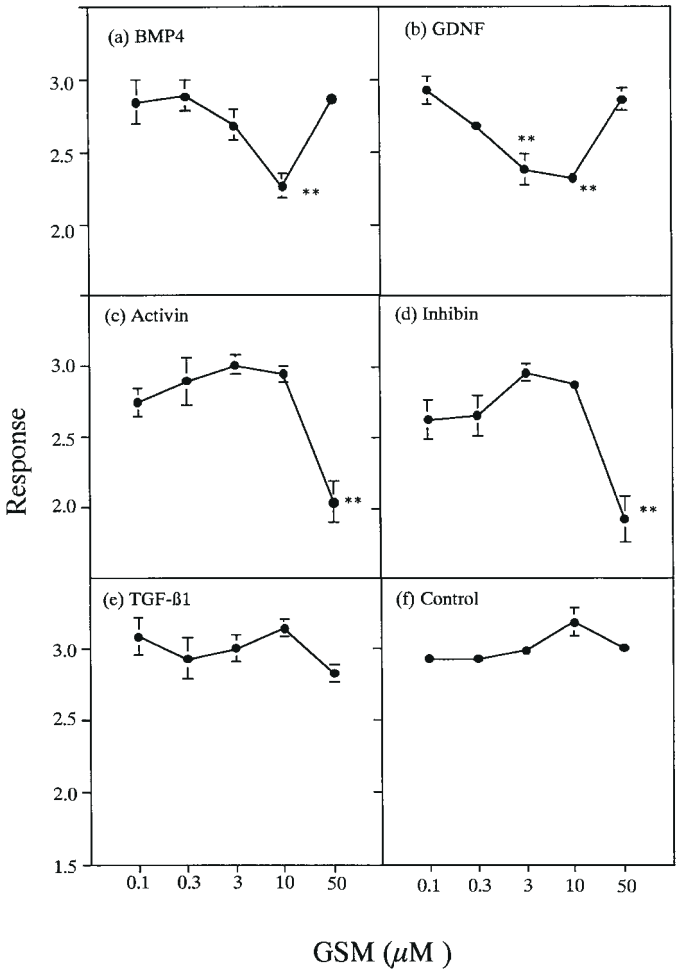

Figure 9 TBF induced by GSM at various concentrations (response) in the presence of a member of the TGF-β1 superfamily (1 ng). Control response was observed in the presence of diluent, 0.2% Prionex (1 μl) alone. Values are means ± SEM (n = 5–6). **Significantly different from the control response by Student's t-test ($P < 0.01$).

effect, indicating that latent TGF-β1 was ineffective. The strong suppression of 0.3 μM GSM-induced TBF by the simultaneous application of CSF and latent TGF-β1 (Figure 8) may be an artifact derived from different batches of CSF samples in the control.

Effect of members of TGF-β superfamily on GSM-induced TBF

TGF-β did not suppress GSM-induced TBF, while other TGF-β superfamily peptides showed a suppressive effect (Figure 9). TBF induced by 10 μM GSM was suppressed by BMP4 at >5.8 fM and that induced by 3 and 10 μM GSM by 1.7 pM and 0.17 fM, respectively, that induced by 50 μM GSM by 0.2 fM activin and that induced by 50 μM GSM by 1.6×10^{-3} aM inhibin (Figure 10). None of the TGF-β superfamily peptides reduced the suppressive effect of rat CSF on GSM-induced TBF (data not shown).

Discussion

In this study, the conditions necessary for GSM-induced TBF were examined in detail. *Hydra*, fed on *Artemia* which had been hatched in a salt solution supplemented with $ZnCl_2$, clearly showed TBF in response to GSM after trypsin treatment. The response to GSM after trypsin treatment was modulated by a number of biologically active peptides.

Direct addition of $ZnCl_2$ to the culture medium was poisonous to *Hydra* at higher concentrations and ineffective at lower concentrations (unpublished observations). It was effective only when $ZnCl_2$ was given through food, though we did not examine the $ZnCl_2$ level in *Artemia*. It is noteworthy that Zn deficiency leads to hypogeusia in human (Atkin-Thor *et al.*, 1978), although the mechanism of Zn requirement common to both humans and *Hydra* is unknown at present. A Zn-metalloprotein, gustin, from the human parotid gland has been reported to be carbonic anhydrase IV and to be correlated with the loss of the sense of taste (Thatcher *et al.*, 1998). It is also interesting to note that incubation of *Hydra* with a bicarbonate solution after trypsin treatment is important in eliciting TBF (unpublished observation).

Many biologically active substances, such as nitric oxide (Colasanti *et al.*, 1995, 1997), arachidonic acid and eicosanoids (Pierobon *et al.*, 1997) have been reported to modulate the feeding response of *Hydra*. The effect of peptides on the response to GSM is outstanding: a large number of biologically active peptides seem to suppress the response to GSM in a specific way depending on individual peptides. Other peptides that were not examined in this study would also suppress the response to GSM. On the other hand, the low mol. wt classical transmitter substances, such as acetylcholine (Erzen and Brzin, 1978), biogenic amines (Hanai and Kitajima, 1984; Ventulini and Carolei, 1992) and γ-aminobutyric acid (Concas *et al.*, 1998) have little to no effect on the response to GSM. It appears that *Hydra*, an animal with one of the most primitive nervous systems, extensively uses peptides as information-transmitting substances. The *Hydra* nervous system has been visualized by a number of antibodies to biologically active peptides (Grimmelikhuijzen and Westafall, 1995). Takahashi *et al.* (Takahashi *et al.*, 1997) reported abundant peptides from *Hydra* tissues that were, potentially, biologically active.

The suppression-nullifying effect of TGF-β was unique among the peptides examined. None of the peptides examined, including members of the TGF-β superfamily, showed a similar effect. TGF-β isoforms have diverse biological activities, including the regulation of cell proliferation and differentiation, stimulation of matrix formation, regulation of cell migration and stimulation of adhesion molecule expression (Massague *et al.*, 1992). Furthermore, TGF-β plays an important role as a modulator of the immune reaction, a mediator of tissue repair in bone formation and

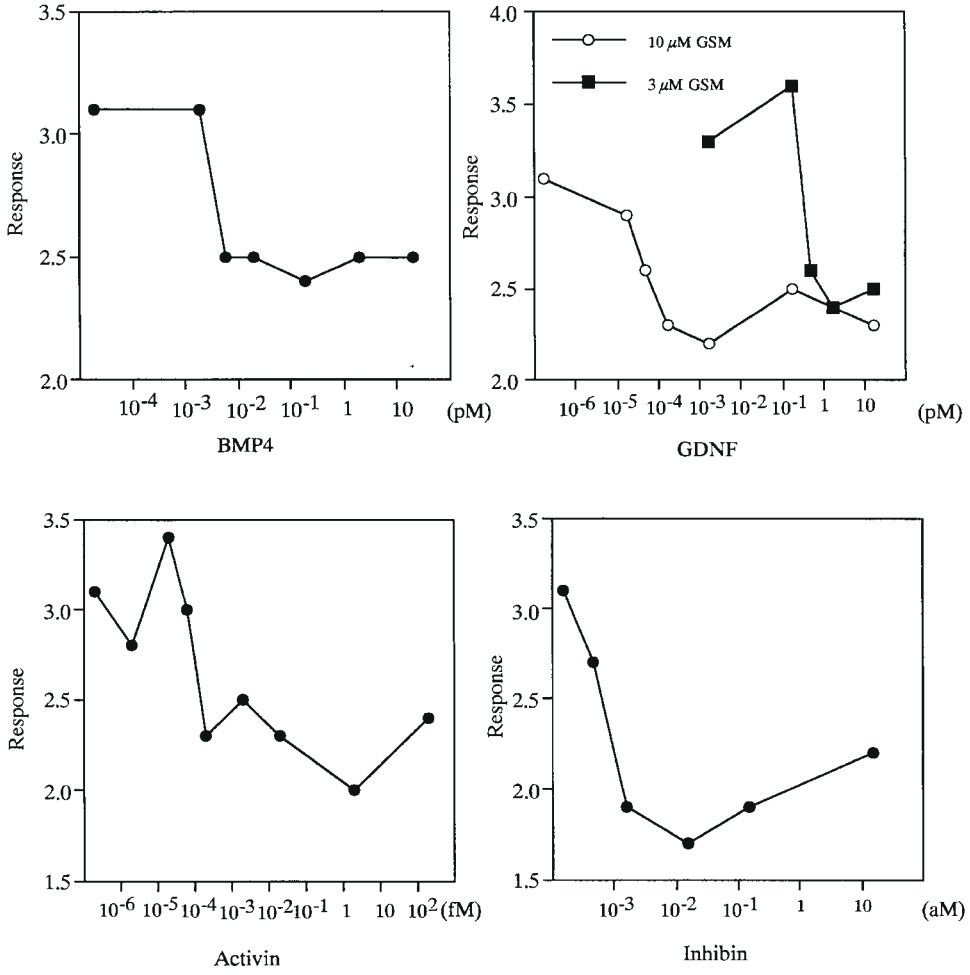

Figure 10 Concentration-dependent effects of BMP4, GDNF, activin and inhibin on GSM-induced TBF (response). **(a)** BMP4 was added together with 10 μM GSM. **(b)** GDNF with 3 or 10 μM GSM. **(c)** Activin with 50 μM GSM. **(d)** Inhibin with 50 μM GSM.

remodeling and processing of wound healing (Barnard *et al.*, 1990; Grande, 1997; Clark and Coker, 1998; O'Kane and Ferguson, 1997). At present we do not understand why mammalian TGF-β modified the response to GSM in *Hydra*. It is likely that *Hydra*, one of the most primitive organisms, has a system influenced by primordial TGF-β. This unique effect of TGF-β may be related to a mechanism involved in the suppression of the response to GSM as well as the possibly unique structural features of TGF-β itself (Schlunegger and Grutter, 1992).

The sensitive, specific modulation of the response to GSM by biologically active peptides should be useful in the study of these peptides.

Acknowledgements

The authors thank Dr Naoto Ueno for his kind gift of BMP4. This study was supported in part by a grant-in-aid from the Ministry of Education, Culture and Science (10640671), and by the 'Research for the Future' Program (JSPS-RFTF97L00906).

References

Atkin-Thor, E., Goddard, B.W., O'Nion, J., Stephen, L. and **Kolff, W.J.** (1978) *Hypogeusia and zinc*. Am. J. Clin. Nutr., 31, 1948–1951.

Barnard, J.A., Lyons, R.M. and **Moses, H.L.** (1990) *The cell biology of transforming growth factor beta*. Biochim. Biophys. Acta, 1032, 79–87.

Clark, D.A. and **Coker, R.** (1998) *Transforming growth factor-beta (TGF-beta).* Int. J. Biochem. Cell Biol., 30, 293–298.

Colasanti, M., Lauro, G.M. and **Ventulini, G.** (1995) *NO in hydra feeding response.* Nature, 374, 505.

Colasanti, M., Ventulini, G., Merante, A., Musci, G. and **Lauro, G.M.** (1997) *Nitric oxide involvement in* Hydra vulgaris *very primitive olfactory-like system*. J. Neurosci., 17, 493–499.

Concas, A., Pierobon, P., Mostallino, M.C., Porcu, P., Marino, G., Minei, R. and **Biggio, G.** (1998) *Modulation of γ-aminobutyric acid (GABA) receptors and the feeding response by neurosteroids in* Hydra vulgaris. Neuroscience, 85, 979–988.

Erzen, I. and **Brzin, M.** (1978) *Cholinergic mechanisms in hydra*. Comp. Biochem. Physiol., 59C, 39–43.

Grande, J.P. (1997) *Role of transforming growth factor-beta in tissue injury and repair*. Proc. Soc. Exp. Biol. Med., 214, 27–40.

Grimmelikhuijzen, C. and **Westafall, J.A.** (1995) *The nervous systems of Cnidarians*. In Breidbach, O. and Kutsch, W. (eds), The Nervous Systems of Invertebrates: an Evolutionary and Comparative Approach. Birkhauser, Basel, pp. 7–24.

Hanai, K. (1998) In situ *potentiation of the glutathione-binding protein for the tentacle ball formation by a protease and efficient ingestion of prey in* Hydra. Comp. Biochem. Physiol., 199A, 333–339.

Hanai, K. and **Kitajima, M.** (1984) *Two types of surface amine receptors modulating the feeding response in* Hydra japonica*: the depressing action of dopamine and related amines*. Chem. Senses, 9, 355–367.

Hanai, K. and **Matsuoka, Y.** (1995) *Potentiation of tentacle ball formation by a trypsin-like protease and accompanying augmented ingestion in glutathione-induced feeding in* Hydra. Zool. Sci., 12, 185–193.

Hanai, K., Kato, H., Matsuhashi, S., Morita, H., Raines, E.W. and **Ross, R.** (1987) *Platelet proteins, including platelet-derived growth factor, specifically depress a subset of the multiple components of the response elicited by glutathione in* Hydra. J. Cell Biol., 104, 1675–1681.

Hanai, K., Oomura, Y., Kai, Y., Nishikawa, K., Shimizu, N., Morita, H. and **Plata-Salaman, C.** (1989) *Central action of acidic fibroblast growth factor in feeding regulation*. Am. J. Physiol., 256, R217–R223.

Inoue, K., Yamazaki, H., Manabe, Y., Fukuda, C., Hanai, K. and **Fushiki, T.** (1999) *Transforming growth factor-beta activated during exercise in brain depresses spontaneous motor activity of animals. Relevance to central fatigue*. Brain Res., 846, 145–153.

Massague, J. (1990) *The transforming growth factor-beta family*. Annu. Rev. Cell Biol., 6, 597–641.

Massague, J., Cheifetz, S., Laiho, M., Ralph, D.A., Weis, F.M. and **Zentella, A.** (1992) *Transforming growth factor-beta*. Cancer Surv., 12, 81–103.

Nishimatsu, S., Suzuki, A., Shoda, A., Murakami, K. and **Ueno, N.** (1992) *Genes for bone morphogenetic proteins are differentially transcribed in early amphibian embryos*. Biochem. Biophys. Res. Commun., 186, 1487–1495.

O'Kane, S. and **Ferguson, M.W.** (1997) *Transforming growth factor betas and wound healing*. Int. J. Biochem. Cell Biol., 29, 63–78.

Pierobon, P., Petrocellis, L., Minei, R. and **Di Marzo, V.** (1997) *Arachidonic acid and eicosanoids in* Hydra*: possible endogenous signals involved in chemoreception and modulation of the feeding behavior*. Adv. Exp. Med. Biol., 433, 363–366.

Schlunegger, M.P. and **Grutter, M.G.** (1992) *An unusual feature revealed by the crystal structure at 2.2 Å resolution of human transforming growth factor-beta 2*. Nature, 358, 430–434.

Takahashi, T., Muneoka, Y., Lohrmann, J., de Hazo, M.S., Sollerder, G., Bosch, T.C., David, C.N., Bode, H.R., Koizumi, O., Shimizu, H., Hatta, M., Fujisawa, T. and **Sugiyama, T.** (1997) *Systematic isolation of peptide signal molecules regulating development in* Hydra*: LW amide and PW families*. Proc. Natl Acad. Sci. USA, 94, 1241–1246.

Thatcher, B.J., Doherty, A.E., Orvisky, E., Martin, B.M. and **Henkin, R.I.** (1998) *Gustin from human parotid saliva is carbonic anhydrase VI*. Biochem. Biophys. Res. Commun., 250, 635–641.

Ventulini, G. and **Carolei, A.** (1992) *Dopaminergic receptors in* Hydra*. Pharmacological and biochemical observations*. Comp. Biochem. Physiol., 102C, 39–43.

Accepted November 18, 1999