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# Royal Jelly Acid, 10-Hydroxy-*trans*-2-Decenoic Acid, as a Modulator of the Innate Immune Responses

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**Abstract:** Royal jelly is a food for queen and larvae honeybees. 10-Hydroxy-*trans*-2-decenoic acid (10H2DA; "royal jelly acid") is the principal lipid component in royal jelly. Several pharmacological activities of 10H2DA have been reported: anti-tumor, anti-biotic, immunomodulatory, estrogenic and neurogenic. We recently revealed an inhibitory effect of 10H2DA in innate immune signals. Despite appreciable advances in studies on innate immune signals after the identification of Toll-like receptors as innate immune receptors, few studies have reported the effect of 10H2DA on innate immune signals. In this review, we focus on recent advances in the evaluation of the biological activities of 10H2DA (especially immunomodulatory activities). We also discuss the molecular mechanisms underpinning these biological activities, which could lead to new therapeutic targets for the treatment of immune disorders.

**Keywords:** Fatty acids, immune disorders, immunomodulator, innate immunity, royal jelly, Toll-like receptors.

## INTRODUCTION

Honey, royal jelly and propolis are sold commercially as "honeybee products". They are also ingredients in certain health foods and have been used as traditional medicines for centuries [1, 2]. Worker honeybees collect nectar from plants and store it as honey in their hives. Royal jelly is a secretion of the hypopharyngeal gland and mandibular gland of young adult worker honeybees [3]. The composition of royal jelly is independent of the environment surrounding beehives (although it may be dependent upon the honeybee species and the ontogeny).

All honeybee larvae are fed some royal jelly for the first 3 days of life [4]. Thereafter, most of the female honeybees become worker bees, whereas a selected female bee fed only royal jelly throughout her life becomes a queen bee. Therefore, royal jelly is critical for the nutritional trans-formation of an immature female larva into a fertile queen bee. Major royal jelly protein (MRJP)1 is a protein component of royal jelly and is also called royalactin. MRJP1 was recently found to play a central part in the development of the honeybee queen [5]. Juvenile hormones may also contribute to the development of queen-like characteristics and caste differentiation in honeybees [6].

Royal jelly is complex mixture of sugars, proteins, lipids, vitamins and minerals (Fig. **1**) [3, 7, 8]. Royal jelly contains a family of proteins called major royal jelly proteins (MRJPs). They constitute ≈90% of the total royal jelly protein [9, 10]. Lipid components are ≈10% of the dry matter in royal jelly. 10-Hydroxy-*trans*-2-decenoic acid (10H2DA) is often referred to as "royal jelly acid". 10H2DA is a major and unique lipid component [11]. About 50% of the total fatty acid content of royal jelly is 10H2DA [11, 12] (Fig. **2**). Other bee products do not contain 10H2DA, so the presence of 10H2DA can be used as a marker to validate the quality of royal jelly from other bee products [13, 14]. 10-Hydroxydecanoic acid (10HDA) is the saturated counterpart of 10H2DA. It is also found in royal jelly as the second most dominant lipid component [11] (Fig. **2**). The biosynthesis of these ω-hydroxy lipids has been reported, and suggests that stearic acid (C18:0) is oxidized at the ω position, shortened to the 10-carbon acid, which is followed by β oxidation [15, 16].

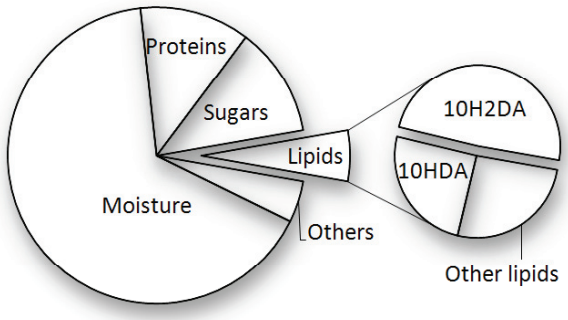


**Fig. (1). Composition of royal jelly.** Major components of royal jelly are as follows: 60-70% moisture content, 9-18% proteins, 7-18% sugars and 3-8% lipids. Other contents are free amino acids, vitamins, salts *etc*. [7, 8, 12]. Most of lipid contents are free fatty acids (90-95%), and 10-hydroxy-*trans*-2-decenoic acid (10H2DA) and 10-hydroxydecanoic acid (10HDA) together present >60-80% [11].

Various pharmacological activities of royal jelly have been reported: anti-tumor, anti-inflammatory and anti-bacterial [17-20]. There are some reports suggesting that

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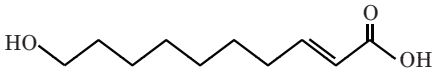
10-Hydroxy-*trans*-2-decenoic acid (10H2DA)




10-Hydroxydecanoic acid (10HDA)

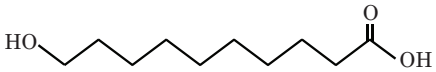



**Fig. (2).** Chemical structures of 10-hydroxy-*trans*-2-decenoic acid (10H2DA) and 10-hydroxydecanoic acid (10HDA).

MRJPs are involved in the biological activity of royal jelly, such as immunomodulatory activity [21] and anti-bacterial activity [22]. 10H2DA is a lipid component found specifically in royal jelly, so several types of biological activity have been investigated (Table **1**).

We will focus here on the recent advances in the evaluation the biological activities of 10H2DA (especially immunomodulatory activities) and discuss the potential underlying molecular mechanisms.

## IMMUNOMODULATORY ACTIVITIES OF 10H2DA IN ROYAL JELLY

Activities of royal jelly on innate and adaptive immunity in *in vitro* and *in vivo* studies have been reported [17]. Furthermore, royal jelly may be beneficial for the treatment of autoimmune and inflammatory diseases [23-25]. It has been revealed that 10H2DA is involved in some of those activities, including inhibition of lipopolysaccharide (LPS)- and interferon (IFN)-γ-stimulated macrophage responses [26-28], inhibition of T-cell proliferation [29, 30] and anti-rheumatoid activity [31, 32].

### Inhibition of Innate Immune Responses

Toll-like receptors (TLRs) and families of cytoplasmic receptors such as nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are known to act as

**Table 1. Molecular mechanisms underpinning the biological activities of 10H2DA.**

| Biological Activity | Molecular Mechanism | Reference |
|---|---|---|
| ***Immunomodulatory*** | | |
| Inhibition of LPS-induced IL-6 production | Inhibition of IκB-ζ induction | [26] |
| Inhibition of LPS-induced NO production | Inhibition of NF-κB activation | [28] |
| Inhibition of IFN-γ-induced NO production | Inhibition of IRF-8 induction | [27] |
| Inhibition of T-cell proliferation | Inhibition of IL-2 production and IL-2 receptor expression | [30] |
| ***Anti-rheumatoid arthritis*** | | |
| Inhibition of TNF-α-induced production of MMPs | Inhibition of p38 and JNK activation | [46] |
| ***Estrogen-like*** | | |
| Activation of estrogen response element | Binding and activation of estrogen receptor | [53] |
| Antagonizing of 17β-estradiol | Modulation of binding affinity of estrogen to estrogen receptor | [54] |
| ***Anti-angiogenesis*** | | |
| Inhibition of VEGF-induced proliferation, migration and tube formation in HUVECs | Possibly inhibition of MMPs | [61] |
| ***Promotion of collagen production*** | | |
| Promotion of collagen production | Induction of TGF-β | [63] |
| ***Epigenetic regulation*** | | |
| Reactivation of epigenetically silenced Fas gene expression | Inhibition of HDAC | [64] |
| ***Modulation of ion channels*** | | |
| Activation of a cation channel, TRPA1, and increase $Ca^{2+}$ permeability | Agonistic activity on TRPA1 | [69] |
| ***Neurogenic*** | | |
| Stimulation of neurogenesis of neural stem/progenitor cells | Possibly BDNF-like activity | [72] |

10H2DA, 10-hydroxy-*trans*-2-decenoic acid; LPS, lipopolysaccharide; IL, interleukin; IκB, inhibitor of nuclear factor-κB; NO, nitric oxide; NF, nuclear factor; IFN, interferon; IRF, interferon-regulatory factor; JNK, c-Jun N-terminal kinase; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; HUVEC, human umbilical vein endothelial cell; TGF, transforming growth factor; HDAC, histone deacetylase; TRPA, transient receptor potential ankyrin; BDNF, brain-derived neurotrophic factor.

major innate immune receptors recognizing microbe-associated molecular patterns. These patterns include LPS from Gram-negative bacteria, bacterial flagella, microbial unmethylated CpG DNA motifs and viral single- or double-stranded RNAs [33]. All of the innate immune signals mediated by TLRs activate nuclear factor (NF)-κB, which is an important transcription factor in innate immune responses. TLR3, 4, 7 and 9 activate transcription of type-I IFN and IFN-related genes. Innate immune responses may contribute to several immune disorders, including inflammatory diseases, autoimmune diseases and allergy [34].

Macrophage activation is one of the initiating events of the innate immune response. The inhibitory effects of royal jelly upon macrophage responses have been reported by Kohno *et al.,* [19]. They examined the effect of separated fractions of royal jelly on LPS- and IFN-γ-stimulated production of tumor necrosis factor (TNF)-α and interleukin (IL)-6 from murine peritoneal macrophages or a murine macrophage-like cell line. Several fractions (including a low-molecular-weight fraction of <5 kDa that was supposed to contain 10H2DA) showed inhibitory effects on the production of TNF-α and/or IL-6; in addition, MRJP3 inhibited the production of TNF-α, but not the production of IL-6.

Recently, we reported that 10H2DA inhibits LPS-induced IL-6 production [26]. 10H2DA does not affect LPS-induced pro-inflammatory cytokines and chemokines other than IL-6. The NF-κB reporter gene assay showed inhibition of TLR-dependent NF-κB activation by 10H2DA, but no effect on the transcription of certain NF-κB-dependent genes. Interestingly, 10H2DA reduced LPS-induced expression of IκB-ζ (a transcription factor activating the κB promoter sequence and which is essential for LPS-induced IL-6 production). The transcription of IκB-ζ is dependent upon NF-κB. Inhibition of the induction of IκB-ζ by 10H2DA was confirmed by reduced expression of IκB-ζ-dependent genes such as lipocalin 2 and granulocyte-colony stimulating factor. From these results, we speculate that 10H2DA inhibits a subset of NF-κB that activates the IκB-ζ promoter and that may be regulated by a post-translational modification of NF-κB subunits.

Nitric oxide (NO) is an important effector molecule that attacks intracellular microbes in macrophages. NO production is also inhibited by 10H2DA [27, 28]. In murine macrophages, LPS induces INF-β production, and its autocrine stimulation is essential for the induction of inducible NO synthase (iNOS), which is responsible for a considerable amount of NO production in macrophages. 10H2DA does not affect LPS-induced IFN-β production or activation of signal transducer and activator of transcription (STAT)1 and STAT2 after autocrine stimulation of IFN-β [28]. IFN-β activates NF-κB through phosphatidylinositole-3 kinase and induces TNF-α production [35]. 10H2DA inhibits NF-κB activation by IFN-β or TNF-α and partially inhibits IFN-β-induced TNF-α production [28]. These findings suggest that inhibition of the activation of NF-κB is responsible for the 10H2DA-mediated inhibition of the induction of iNOS. IFN-γ also induces NO production after activation of STAT1 and the production of TNF-α followed by NF-κB activation [36]. IFN-γ induces the *de novo* production of interferon regulatory factor (IRF)-1 and IRF-8, both of which are necessary for the transcription of TNF-α in IFN-γ stimulation [37]. 10H2DA inhibits the induction of IRF-8 (but not IRF-1), followed by the inhibition of TNF-α production, whereas STAT1 activation is not affected [27]. Autocrine stimulation of TNF-α is responsible for NF-κB activation during IFN-γ stimulation, so inhibition of TNF-α production by 10H2DA results in the inhibition of NF-κB activation. Inhibition of IFN-γ-induced iNOS production by 10H2DA has been postulated to be a result of the inhibition of NF-κB activation.

Duplan *et al*. also reported the inhibition of TLR signaling by 10H2DA [38]. They prepared samples of reconstructed human epidermis and stimulated them with polyinosinic-polycytidylic acid (poly I:C) and IL-1. Poly I:C is a synthetic ligand of TLR3 mimicking double-stranded RNAs. The IL-1 receptor contains a Toll/IL-1 receptor domain in the cytoplasmic tail and activates the same signaling cascades as TLRs [39]. Stimulation of poly I:C and IL-1 activated NF-κB and induced thymic stromal lymphopoietin (TSLP) [40]. 10H2DA decreased poly I:C- and IL-1-induced TSLP release from the reconstructed epidermis [38]. They also revealed the inhibitory effect of 10H2DA on the release of IL-4, IL-5 and IL-13 from inflamed and damaged skin explants. IL-4, IL-5 and IL-13 are Th2 cytokines that upregulate IgE production. Therefore, 10H2DA has been postulated to influence not only the innate immune system but also the adaptive immune system.

## Modulation of Adaptive Immunity

Several reports have demonstrated the effect of royal jelly on antibody production, which can be related to anti-allergic effects. Sver *et al*. showed that royal jelly exhibited immune stimulatory effects by enhancing antibody production and proliferation of immunocompetent cells in mice after immunization with the red blood cells of sheep [41]. Conversely, they also reported the immunosuppressive effects of royal jelly on T cell-mediated antibody production in rats.

Suppression of type-I allergic reactions by royal jelly have been reported. Oka *et al.* reported that oral administration of royal jelly suppressed antigen-specific IgE production and histamine release from mast cells in association with the restoration of macrophage function and improvement of Th1/Th2 cell responses in 2,4-dinitrophenylated keyhole limpet hemocyanin (DNP-KLH)-immunized mice [42]. Taniguchi *et al.* reported that oral administration of royal jelly inhibited the development of atopic dermatitis lesions in picryl chloride-treated NC/Nga mice [43]. Kataoka and colleagues demonstrated that intraperitoneal administration of royal jelly into ovalbumin (OVA)/alum-immunized mice resulted in the reduction of OVA-specific IgE antibody in the sera as well as IL-4, IL-5, IL-10 and IFN-γ production by OVA-stimulated spleen cells, suggesting downregulation of Th1 and Th2 responses [44]. MRJP3 has been described as a candidate dominant immunomodulator suppressing cytokine production (especially IL-4) from T cells and thereby promoting anti-allergic responses [21].

The immunomodulatory effect of 10H2DA upon adaptive immunity has been reported by Colic and colleagues. They extracted and isolated several components of royal jelly [45], then examined T-cell proliferation *in vitro* [29]. One fraction, MEL 138 (which contains 10H2DA) showed a slight (but significant) inhibitory effect upon concanavalin A-stimulated splenocyte proliferation, but IL-2 production was not affected. Furthermore, they showed that 10H2DA and 3,10-dihydroxydecanoic acid, isolated from royal jelly, inhibited T-cell proliferation by using dendritic cell (DC)-T-cell cocultures [30]. The effect of 10H2DA was followed by a decrease in IL-2 production and down-regulation of expression of the IL-2 receptor on T cells. Antigen-specific antibody responses were also suppressed by 3,10-dihydroxydecanoic acid *in vivo*.

### Anti-rheumatoid Arthritis (RA) Activity

RA is a complex immune-mediated disease of unknown etiology. Evidence for the beneficial effects of 10H2DA upon RA was provided by Wang and colleagues. They examined the effect of 10H2DA on TNF-α responses of synovial fibroblasts isolated from rheumatoid tissues of RA patients [31]. 10H2DA inhibited TNF-α-induced expression of matrix metalloproteinases (MMPs), which are critical proteases involved in tissue degradation in RA [46]. TNF-α-induced phosphorylation of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) were inhibited by 10H2DA, although NF-κB activation and phosphorylation of extracellular signal-regulated kinase (ERK) were not affected. They also reported that 10H2DA downregulated expression of connective tissue growth factor followed by downregulation of the expression of MMPs [32].

## OTHER BIOLOGICAL ACTIVITIES OF 10H2DA

### Estrogen-like Activity: 10H2DA as an Agonist and/or Antagonist of the Estrogen Receptors

Traditionally, royal jelly has been used to improve post-menopausal symptoms [1]. Clinical studies have revealed the beneficial effects of royal jelly on autonomic imbalance in menopausal women [47, 48]. Loss of bone mass is one of the major symptoms in post-menopausal women. Studies to verify the preventative effects of royal jelly on osteoporosis have been conducted. Royal jelly prevented ovariectomy-induced bone loss in rats, and this protective effect was as effective as that produced by 17β-estradiol [49]. Protease-treated royal jelly also showed an equivalent effect to that of raw royal jelly, suggesting that the active materials in royal jelly could be protease-insensitive. Royal jelly also stimulates bone formation *in vitro* and *in vivo* [50]. In the same study, royal jelly stimulated not only cell proliferation but also collagen production in murine osteoblast-like cells.

Mishima *et al*. showed that royal jelly has estrogenic activities *in vitro* and *in vivo* [51, 52]. Royal jelly enhanced proliferation of the estrogen-sensitive breast cancer cell line MCF-7, and upregulated gene transcriptions that were dependent upon the estrogen responsive element. Some components in royal jelly were shown to interact with estrogen receptors. They further elucidated that 10H2DA is a critical component expressing estrogenic activities.

Suzuki *et al*. reported the estrogenic activities of certain lipids in royal jelly: 10H2DA, 10HDA, *trans*-2-decenoic acid and 24-methylenecholesterol [53]. These lipids activate the estrogen responsive element-dependent transcription and proliferation of MCF-7 cells in an estrogen receptor-dependent manner. They also showed competitive inhibition of the binding of 17β-estradiol to human estrogen receptor β (but not to estrogen receptor α) by these lipids. Subcutaneous injection of any of these lipids into immature rats induced mild hypertrophy of the luminal epithelium of the uterus.

The agonistic and antagonistic activities of fatty acids derived from royal jelly have been investigated using estrogen receptor α- and/or β-expressing cell lines [54]. 10H2DA, 3,10-dihydroxydecanoic acid and sebacic acid were isolated from royal jelly and examined. These fatty acids showed weak agonistic activity in estrogen receptor β- (but not α-) expressing MCF-7 cells in the absence of 17β-estradiol. Conversely, these fatty acids were agonistic in estrogen receptor α- (but not β-) expressing HeLa cells. In the presence of 17β-estradiol, however, these fatty acids antagonized 17β-estradiol-induced transactivation of estrogen responsive element in estrogen receptor α- and β-expressing cells. They found that 10H2DA altered 17β-estradiol-induced co-activator recruitment to estrogen receptor α. Although the weak binding of 10H2DA (but not of the other lipids) to the ligand-binding domains of estrogen receptor α and β was detected, they speculated that these fatty acids induce a conformational change in estrogen receptors, resulting in modulation of the recruitment of estrogen receptors and co-activators to the target genes.

### Anti-tumor Activity

Anti-tumor activity of 10H2DA was first reported in 1959 [55, 56] with demonstration of inhibition of the growth of tumor cells *in vitro*. Several reports have shown that royal jelly inhibits the growth and/or metastasis of tumor cells [57]. Tamura *et al.* showed that royal jelly administered *via* the oral route inhibited the growth of slow-growing tumors (*e.g.*, Ehrlich ascites, sarcoma-180 ascites) in rats [18]. Bincoletto *et al*. also reported the effectiveness of royal jelly on Ehrlich ascites tumor-bearing mice with extension of survival at higher doses [58]. Orsolic *et al*. reported that royal jelly did not affect the formation of metastases if administered *via* the intraperitoneal or subcutaneous route. However, synchronous intravenous application of tumor cells and royal jelly significantly inhibited the formation of metastases [59, 60].

Angiogenesis is required for invasive tumor growth and metastasis. Angiogenesis constitutes an important point in the control of cancer progression. Anti-angiogenesis activity of 10H2DA has been reported by Izuta *et al.,* [61]. Vascular endothelial growth factor (VEGF)-induced proliferation, migration and tube formation in human umbilical vein endothelial cells (HUVECs) was inhibited by 10H2DA. The MMP inhibitor GM6001 similarly prevented VEGF-induced

migration and tube formation in HUVECs. Hence, they speculated that MMPs are inhibited by 10H2DA. It may also be possible to inhibit the expression of MMPs by 10H2DA, as described above.

### Promotion of Collagen Production

Koya-Miyata *et al*. showed that royal jelly increases collagen production from fibroblast cells [62]. The activity was found in the alkali-soluble fraction of royal jelly, and 10H2DA was identified as an active component [63]. Royal jelly and 10H2DA induced the production of transforming growth factor (TGF)-β, which is known to be an important inducer of collagen production. Involvement of TGF-β in 10H2DA-promoted collagen production was confirmed by anti-TGF-β antibody, by which the promotion was completely inhibited. Notably, 10HDA also showed similar promotion of collagen production to that seen with 10H2DA.

### Epigenetic Regulatory Activity

Spannhoff *et al.* reported the histone deacetylase inhibitor (HDACi) activity of 10H2DA [64]. In K-ras-transformed NIH3T3 cells, the Fas gene is epigenetically silenced by the activated Ras pathway [65, 66]. 10H2DA reactivated the silenced gene expression. The HDACi activity of 10H2DA was confirmed in an *in vitro* assay, while inhibition of DNA methylation was not observed.

### Modulation of Ion Channels

Transient receptor potential (TRP) ankyrin (TRPA)1 is a $Ca^{2+}$ permeable non-selective cation channel belonging to the TRP family [67, 68]. Terada *et al*. examined the effect of royal jelly on TRPA1 activity [69]. They found that hexane extracts and ethyl acetate extracts of lyophilized royal jelly increased $Ca^{2+}$ permeability in TRPA1-expressing HEK293 cells. They identified 10H2DA and 10HDA as the major components in royal jelly that activate TRPA1.

### Neurogenic Activity

Neuronal activities of royal jelly and/or 10H2DA have been investigated *in vitro* and *in vivo* by Furukawa and colleagues [70]. Oral administration of royal jelly showed neurotrophic effects on the mature brains of ddY mice *via* stimulation of glial cell line-derived neurotrophic factor (GDNF) production, and that enhanced expression of neurofilament H was involved in the events subsequently caused by GDNF [71]. *In vitro* experiments showed that 10H2DA stimulated neurogenesis (but suppressed gliogenesis) in neural stem/progenitor cells, whereas royal jelly facilitated the differentiation of all types of brain cells (including neurons, astrocytes and oligodendrocytes) [72]. Furthermore, intraperitoneal administration of 10H2DA showed protective effects against models of stress-induced depression and anxiety in mice [73]. The nature of the intracellular signals of 10H2DA that regulate differentiation of neuronal stem/progenitor cells is not known, but they speculated that 10H2DA partly mimics the effect of brain-derived neurotrophic factor (BDNF) [72].

## POSSIBLE MECHANISMS UNDERPINNING THE IMMUNOMODULATORY ACTIVITY OF 10H2DA

10H2DA and 10HDA show comparable effects with respect to: estrogenic activity; *in vitro* anti-tumor activity; promotion of collagen production; and TRPA1 channel activation. We could not detect the 10H2DA-like inhibitory effect of 10HDA on LPS-induced IL-6 production (unpublished data). Other fatty acids and sterols show similar activity to 10H2DA, such as the: inhibitory activity of 3,10-dihydroxydecanoic acid on DC-dependent T-cell proliferation [30]; estrogenic activity of *trans*-2-decenoic acid, 24-methylenecholesterol, 3,10-dihydroxydecanoic acid and sebacic acid [53, 54]; inhibitory activity of 3,10-dihydroxydecanoic acid on concanavalin A-stimulated splenocyte proliferation [29]; and TRPA1 channel activation by C8–10 medium-chain fatty acids derived from royal jelly. Comparison of the biological activities among these structurally related compounds may suggest the relationship between the mechanisms of action of 10H2DA and 10HDA.

Specific inhibitors against a signaling cascade bind directly to one of the signaling molecules and inhibit its enzymatic activity or interaction with another signaling molecule. Reports demonstrating direct evidence of 10H2DA binding to any of the signaling molecules in innate immune signaling cascades are lacking. We speculate that specific inhibition of 10H2DA on LPS-induced IκB-ζ expression may be due to inhibition of post-translational modification (*e.g.*, phosphorylation) of an NF-κB subunit, p65 [26]. Also, 10H2DA may inhibit an upstream signaling molecule which is necessary to activate a subset of the NF-κB complex.

Only estrogenic receptors have been reported to behave as receptors, to which 10H2DA and other related lipids bind directly, as described above. Estrogen receptors are members of a nuclear receptor family, are present intracellularly, and are activated by binding of their ligands followed by activation of target gene transcriptions. Estrogen receptor-dependent activation of the estrogen response element by 10H2DA suggests that 10H2DA interacts intracellularly with an estrogen receptor. Therefore, 10H2DA could interact directly with an intracellular signaling molecule in innate immune signaling cascades and inhibit its signaling activity.

GPR30 is a G protein-coupled receptor (GPCR). It functions as a cell-surface receptor for estrogens [74]. GPR30 signals may act synergistically or antagonize estrogen receptor α-mediated gene expression [75]. With the finding of an antagonistic effect of 10H2DA and other lipids by Moutsatsou *et al.*, they suggested that these lipids could be ligands of GPR30 [54].

Some GPCRs have been reported to be receptors for free fatty acids [76, 77]. GPR120 and GPR40 are activated by medium- and long-chain fatty acids [78, 79], and GPR119 is activated by long-chain fatty acids [80]. GPR84 is activated by medium-chain fatty acids [81], whereas GPR43 and GPR41 are activated by short-chain fatty acids [82]. GPR84 is activated by C9–14 free fatty acids (especially by C10 fatty acids) but the signal may not be related to the inhibitory effect of 10H2DA on LPS-induced IL-6 production because the signal enhances the response to LPS [81]. GPR120 also recognizes ω-3 fatty acids such as α-linolenic acid,

docosahexaenoic acid and eicosapentaenoic acid, and the signal abolishes LPS-mediated activation of NF-κB and JNK in macrophages [83]. Interestingly, ω-3 fatty acids-induced GPR120 signals inhibit LPS-stimulated TNF-α production, which is different from our finding on 10H2DA. Many orphan GPCRs are coded within mammalian genomes, so GPCR signaling may contribute to immunomodulatory activities (including the inhibitory effect of 10H2DA on innate immune signaling).

## CONCLUDING REMARKS

10H2DA is a unique fatty acid found specifically in royal jelly and shows various pharmacological activities. With respect to inhibition of innate immune signals, however, 10H2DA shows a quite restricted effect which is dependent upon the stimulation. Content analyses of royal jelly reveal that the typical concentration of 10H2DA in raw royal jelly should be >100 mM. The 10H2DA concentration that can inhibit innate immune signals is more than a few millimolars, but reaching the effective concentration when administering to the gastrointestinal tract or skin is relatively easy. Furthermore, developing more specific and higher-affinity drugs from 10H2DA as a lead compound should be achievable. Indeed, 2-decenoic acid ethyl ester was found to be a derivative of unsaturated medium-chain fatty acids with a more potent neuroprotective effect [84, 85]. Unfortunately, we did not find a greater inhibitor of innate immune signals in our screening. Several recent studies have revealed that innate immune signals are involved in many autoimmune and inflammatory diseases, but those signals are important for immune responses against infective microbes. Hence, compounds manifesting a higher specificity of inhibition against the restricted innate immune signaling cascade should be necessary for developing drugs against disorders of the innate immune system without exhibiting side effects. 10H2DA could be a unique lead compound for therapeutic drugs against disorders of the innate immune system.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ACKNOWLEDGEMENTS

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## ABBREVIATIONS

| | | |
|---|---|---|
| 10H2DA | = | 10-hydroxy-*trans*-2-decenoic acid |
| 10HDA | = | 10-hydroxydecanoic acid |
| BDNF | = | brain-derived neurotrophic factor |
| DC | = | dendritic cell |
| DNP-KLH | = | 2,4-dinitrophenylated keyhole limpet hemocyanin |
| ERK | = | extracellular signal-regulated kinase |
| GDNF | = | glial cell line-derived neurotrophic factor |
| GPCR | = | G protein-coupled receptor |
| HDAC | = | histone deacetylase |
| HDACi | = | histone deacetylase inhibitor |
| HUVEC | = | human umbilical vein endothelial cell |
| IFN | = | interferon |
| IκB | = | inhibitor of nuclear factor-κB |
| IL | = | interleukin |
| iNOS | = | inducible nitric oxide synthase |
| IRF | = | interferon-regulatory factor |
| JNK | = | c-Jun N-terminal kinase |
| LPS | = | lipopolysaccharide |
| MAPK | = | mitogen-activated protein kinase |
| MMP | = | matrix metalloproteinase |
| MRJP | = | major royal jelly proteins |
| NF | = | nuclear factor |
| NLR | = | nucleotide-binding oligomerization domain-like receptor |
| NO | = | nitric oxide |
| NOD | = | nucleotide-binding oligomerization domain |
| OVA | = | ovalbumin |
| poly I:C | = | polyinosinic-polycytidylic acid |
| RA | = | rheumatoid arthritis |
| RIG | = | retinoic acid-inducible gene |
| RLR | = | retinoic acid-inducible gene-I-like receptor |
| STAT | = | signal transducer and activator of transcription |
| TGF | = | transforming growth factor |
| TLR | = | Toll-like receptor |
| TNF | = | tumor necrosis factor |
| TRP | = | transient receptor potential |
| TRPA | = | transient receptor potential ankyrin |
| VEGF | = | vascular endothelial growth factor |

## REFERENCES

[1] Cherniack, E.P. (2010) Bugs as drugs, Part 1: Insects: the "new" alternative medicine for the 21st century? *Altern. Med. Rev.*, **15**, 124-135.

[2] Miyata, T. (2007) Pharmacological basis of traditional medicines and health supplements as curatives. *J. Pharmacol. Sci.*, **103**, 127-131.

[3] Rembold, H. (1965) Biologically active substances in royal jelly. *Vitam. Horm.*, **23**, 359-382.

[4] Weaver, N. (1955) Rearing of Honeybee Larvae on Royal Jelly in the Laboratory. *Science*, **121**, 509-510.

[5] Kamakura, M. (2011) Royalactin induces queen differentiation in honeybees. *Nature*, **473**, 478-483.

[6] Barchuk, A.R.; Cristino, A.S.; Kucharski, R.; Costa, L.F.; Simoes, Z.L. and Maleszka, R. (2007) Molecular determinants of caste differentiation in the highly eusocial honeybee Apis mellifera. *BMC Dev. Biol.*, **7**, 70.

[7] Sabatini, A.G.; Marcazzan, G.L.; Caboni, M.F.; Bogdanov, S. and Almeida-Muradian, L.B.d. (2009) Quality and standardisation of Royal Jelly. *J. ApiProd. ApiMed. Sci.*, **1**, 16-21.

[8] Melampy, R.M. and Jones, D.B. (1939) Chemical Composition and Vitamin Content of Royal Jelly. *Proc. Soc. Exp. Biol. Med. Soc.*, **41**, 382-388.

[9] Schmitzova, J.; Klaudiny, J.; Albert, S.; Schroder, W.; Schreckengost, W.; Hanes, J.; Judova, J. and Simuth, J. (1998) A family of major royal jelly proteins of the honeybee *Apis mellifera* L. *Cell. Mol. Life Sci.*, **54**, 1020-1030.

[10] Drapeau, M.D.; Albert, S.; Kucharski, R.; Prusko, C. and Maleszka, R. (2006) Evolution of the Yellow/Major Royal Jelly Protein family and the emergence of social behavior in honey bees. *Genome Res.*, **16**, 1385-1394.

[11] Lercker, G.; Capella, P.; Conte, L.S.; Ruini, F. and Giordani, G. (1982) Components of royal jelly II. The lipid fraction, hydrocarbons and sterols. *J. Apic. Res.*, **21**, 178-184.

[12] Howe, S.R.; Dimick, P.S. and Benton, A.W. (1985) Composition of freshly harvested and commercial royal jelly. *J. Apic. Res.*, **24**, 52-61.

[13] Antinelli, J.-F.; Zeggane, S.; Davico, R.; Rognone, C.; Faucon, J.-P. and Lizzani, L. (2003) Evaluation of (E)-10-hydroxydec-2-enoic acid as a freshness parameter for royal jelly. *Food Chem.*, **80**, 85-89.

[14] Weaver, N. and Law, J.H. (1960) Heterogeneity of fatty acids from royal jelly. *Nature*, **188**, 938-939.

[15] Plettner, E.; Sutherland, G.R.J.; Slessor, K.N. and Winston, M.L. (1995) Why not be a queen? Regioselectivity in mandibular secretions of honeybee castes. *J. Chem. Ecol.* **21**, 1017-1029.

[16] Plettner, E.; Slessor, K.N.; Winston, M.L. and Oliver, J.E. (1996) Caste-Selective Pheromone Biosynthesis in Honeybees. *Science*, **271**, 1851-1853.

[17] Pavel, C.I.; Mărghitaş, L.A.; Bobiş, O.; Dezmirean, D.S.; Şapcaliu, A.; Radoi, I. and Mădaş, M.N. (2011) Biological Activities of Royal Jelly - Review. *Scientifical Papers, Anim. Sci. Biotechnol.*, **44**, 108-118.

[18] Tamura, T.; Fujii, A. and Kuboyama, N. (1987) Antitumor effects of royal jelly (RJ). *Folia Pharmacol. Japon.*, **89**, 73-80.

[19] Kohno, K.; Okamoto, I.; Sano, O.; Arai, N.; Iwaki, K.; Ikeda, M. and Kurimoto, M. (2004) Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. *Biosci. Biotechnol. Biochem.*, **68**, 138-145.

[20] Helleu, C. (1956) Antibacterial properties of royal jelly; bactericidal and antibiotic effects of neutralized royal jelly. *Ann. Inst. Pasteur (Paris)*, **91**, 231-237.

[21] Okamoto, I.; Taniguchi, Y.; Kunikata, T.; Kohno, K.; Iwaki, K.; Ikeda, M. and Kurimoto, M. (2003) Major royal jelly protein 3 modulates immune responses *in vitro* and *in vivo*. *Life Sci.*, **73**, 2029-2045.

[22] Fontana, R.; Mendes, M.A.; Souza, B.M.d.; Konno, K.; Cesar, L.M.M.; Malaspina, O. and Palma, M.S. (2004) Jelleines: a family of antimicrobial peptides from the Royal Jelly of honeybees (Apis mellifera). *Peptides*, **25**, 919-928.

[23] Erem, C.; Deger, O.; Ovali, E. and Barlak, Y. (2006) The effects of royal jelly on autoimmunity in Graves' disease. *Endocrine*, **30**, 175-183.

[24] Mannoor, M.K.; Shimabukuro, I.; Tsukamotoa, M.; Watanabe, H.; Yamaguchi, K. and Sato, Y. (2009) Honeybee royal jelly inhibits autoimmunity in SLE-prone NZB x NZW F1 mice. *Lupus*, **18**, 44-52.

[25] Karaca, T.; Bayiroglu, F.; Yoruk, M.; Kaya, M.S.; Uslu, S.; Comba, B. and Mis, L. (2010) Effect of royal jelly on experimental colitis induced by acetic acid and alteration of mast cell distribution in the colon of rats. *Eur. J. Histochem.*, **54**, e35.

[26] Sugiyama, T.; Takahashi, K.; Tokoro, S.; Gotou, T.; Neri, P. and Mori, H. (2011) Inhibitory effect of 10-hydroxy-*trans*-2-decenoic acid on LPS-induced IL-6 production *via* reducing IκB-ζ expression. *Innate Immun.*, **18**, 429-437.

[27] Takahashi, K.; Sugiyama, T.; Tokoro, S.; Neri, P. and Mori, H. (2012) Inhibition of interferon-γ-induced nitric oxide production by 10-hydroxy-*trans*-2-decenoic acid through inhibition of interferon regulatory factor-8 induction. *Cell. Immunol.*, **273**, 73-78.

[28] Sugiyama, T.; Takahashi, K.; Kuzumaki, A.; Tokoro, S.; Neri, P. and Mori, H. (2012) Inhibitory Mechanism of 10-Hydroxy-trans-2-decenoic acid (Royal Jelly Acid) against Lipopolysaccharide- and Interferon-β-induced Nitric Oxide Production. *Inflammation*, in press.

[29] Gasic, S.; Vucevic, D.; Vasilijic, S.; Antunovic, M.; Chinou, I. and Colic, M. (2007) Evaluation of the immunomodulatory activities of royal jelly components *in vitro*. *Immunopharmacol. Immunotoxicol.*, **29**, 521-536.

[30] Vucevic, D.; Melliou, E.; Vasilijic, S.; Gasic, S.; Ivanovski, P.; Chinou, I. and Colic, M. (2007) Fatty acids isolated from royal jelly modulate dendritic cell-mediated immune response *in vitro*. *Int. Immunopharmacol.*, **7**, 1211-1220.

[31] Yang, X.Y.; Yang, D.S.; Wei, Z.; Wang, J.M.; Li, C.Y.; Hui, Y.; Lei, K.F.; Chen, X.F.; Shen, N.H.; Jin, L.Q. and Wang, J.G. (2010) 10-Hydroxy-2-decenoic acid from Royal jelly: a potential medicine for RA. *J. Ethnopharmacol.*, **128**, 314-321.

[32] Wang, J.G.; Ruan, J.; Li, C.Y.; Wang, J.M.; Li, Y.; Zhai, W.T.; Zhang, W.; Ye, H.; Shen, N.H.; Lei, K.F.; Chen, X.F. and Yang, X.Y. (2012) Connective tissue growth factor, a regulator related with 10-hydroxy-2-decenoic acid down-regulate MMPs in rheumatoid arthritis. *Rheumatol. Int.*, **32**, 2791-2799.

[33] Kimura, H.J.; Suzuki, K.; Landek-Salgado, M.A.; Caturegli, P.; Jounai, N.; Kobiyama, K. and Takeshita, F. (2011) Application of innate immune molecules for a new class of drugs: infection, inflammation and beyond. *Endocr. Metab. Immune Disord. Drug Targets*, **11**, 68-75.

[34] Montero Vega, M.T. and de Andres Martin, A. (2009) The significance of toll-like receptors in human diseases. *Allergol. Immunopatholog.*, **37**, 252-263.

[35] Yang, C.H.; Murti, A.; Pfeffer, S.R.; Kim, J.G.; Donner, D.B. and Pfeffer, L.M. (2001) Interferon α/β promotes cell survival by activating nuclear factor κB through phosphatidylinositol 3-kinase and Akt. *J. Biol. Chem.*, **276**, 13756-13761.

[36] Vila-del Sol, V.; Diaz-Munoz, M.D. and Fresno, M. (2007) Requirement of tumor necrosis factor α and nuclear factor-κB in the induction by IFN-γ of inducible nitric oxide synthase in macrophages. *J. Leukoc. Biol.*, **81**, 272-283.

[37] Vila-del Sol, V.; Punzon, C. and Fresno, M. (2008) IFN-γ-induced TNF-α expression is regulated by interferon regulatory factors 1 and 8 in mouse macrophages. *J. Immunol.*, **181**, 4461-4470.

[38] Duplan, H.; Questel, E.; Hernandez-Pigeon, H.; Galliano, M.F.; Caruana, A.; Ceruti, I.; Ambonati, M.; Mejean, C.; Damour, O.; Castex-Rizzi, N.; Bessou-Touya, S. and Schmitt, A.M. (2011) Effects of Hydroxydecine((R)) (10-hydroxy-2-decenoic acid) on skin barrier structure and function *in vitro* and clinical efficacy in the treatment of UV-induced xerosis. *Eur. J. Dermatol.*, **21**, 906-915.

[39] O'Neill, L. (2000) The Toll/interleukin-1 receptor domain: a molecular switch for inflammation and host defence. *Biochem. Soc. Trans.*, **28**, 557-563.

[40] Kinoshita, H.; Takai, T.; Le, T.A.; Kamijo, S.; Wang, X.L.; Ushio, H.; Hara, M.; Kawasaki, J.; Vu, A.T.; Ogawa, T.; Gunawan, H.; Ikeda, S.; Okumura, K. and Ogawa, H. (2009) Cytokine milieu modulates release of thymic stromal lymphopoietin from human keratinocytes stimulated with double-stranded RNA. *J. Allergy Clin. Immunol.*, **123**, 179-186.

[41] Sver, L.; Orsolic, N.; Tadic, Z.; Njari, B.; Valpotic, I. and Basic, I. (1996) A royal jelly as a new potential immunomodulator in rats and mice. *Comp. Immunol. Microbiol. Infect. Dis.*, **19**, 31-38.

[42] Oka, H.; Emori, Y.; Kobayashi, N.; Hayashi, Y. and Nomoto, K. (2001) Suppression of allergic reactions by royal jelly in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses. *Int. Immunopharmacol.*, **1**, 521-532.

[43] Taniguchi, Y.; Kohno, K.; Inoue, S.; Koya-Miyata, S.; Okamoto, I.; Arai, N.; Iwaki, K.; Ikeda, M. and Kurimoto, M. (2003) Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. *Int. Immunopharmacol.*, **3**, 1313-1324.

[44] Kataoka, M.; Arai, N.; Taniguchi, Y.; Kohno, K.; Iwaki, K.; Ikeda, M. and Kurimoto, M. (2001) Analysis of Anti-allergic Function of Royal Jelly. *Nat. Med.*, **55**, 174-180.

[45] Melliou, E. and Chinou, I. (2005) Chemistry and bioactivity of royal jelly from Greece. *J. Agric. Food Chem.*, **53**, 8987-8992.

[46] Green, M.J.; Gough, A.K.; Devlin, J.; Smith, J.; Astin, P.; Taylor, D. and Emery, P. (2003) Serum MMP-3 and MMP-1 and

progression of joint damage in early rheumatoid arthritis. *Rheumatology (Oxford)*, **42**, 83-88.

[47] Kushima, K. and Hasegawa, N. (1972) Menopausal disorder. *J. Ther.*, **54**, 578-584.

[48] Kushima, K.; Hasegawa, N. and Ogawa, E. (1973) Effects of royal jelly on autonomic imbalance in menopausal women. *World Obstet. Gynecol.*, **25**, 439-443.

[49] Hidaka, S.; Okamoto, Y.; Uchiyama, S.; Nakatsuma, A.; Hashimoto, K.; Ohnishi, S.T. and Yamaguchi, M. (2006) Royal jelly prevents osteoporosis in rats: beneficial effects in ovariectomy model and in bone tissue culture model. *Evid. based Complement. Alternat. Med.*, **3**, 339-348.

[50] Narita, Y.; Nomura, J.; Ohta, S.; Inoh, Y.; Suzuki, K.M.; Araki, Y.; Okada, S.; Matsumoto, I.; Isohama, Y.; Abe, K.; Miyata, T. and Mishima, S. (2006) Royal jelly stimulates bone formation: physiologic and nutrigenomic studies with mice and cell lines. *Biosci. Biotechnol. Biochem.*, **70**, 2508-2514.

[51] Mishima, S.; Suzuki, K.M.; Isohama, Y.; Kuratsu, N.; Araki, Y.; Inoue, M. and Miyata, T. (2005) Royal jelly has estrogenic effects *in vitro* and *in vivo*. *J. Ethnopharmacol.*, **101**, 215-220.

[52] Mishima, S.; Miyata, T.; Suzuki, K.; Araki, Y.; Akao, Y. and Isohama, Y. (2005), Estrogenic effects of royal jelly(Chemical & Pharmacological study). *J. Trad. Med.*, **22**, 171-175.

[53] Suzuki, K.M.; Isohama, Y.; Maruyama, H.; Yamada, Y.; Narita, Y.; Ohta, S.; Araki, Y.; Miyata, T. and Mishima, S. (2008) Estrogenic activities of fatty acids and a sterol isolated from royal jelly. *Evid. based Complement. Alternat. Med.*, **5**, 295-302.

[54] Moutsatsou, P.; Papoutsi, Z.; Kassi, E.; Heldring, N.; Zhao, C.; Tsiapara, A.; Melliou, E.; Chrousos, G.P.; Chinou, I.; Karshikoff, A.; Nilsson, L. and Dahlman-Wright, K. (2010) Fatty acids derived from royal jelly are modulators of estrogen receptor functions. *PLoS ONE*, **5**, e15594.

[55] Townsend, G.F.; Morgan, J.F. and Hazlett, B. (1959) Activity of 10-hydroxydecenoic acid from royal jelly against experimental leukaemia and ascitic tumours. *Nature*, **183**, 1270-1271.

[56] Townsend, G.F.; Morgan, J.F.; Tolnai, S.; Hazlett, B.; Morton, H.J. and Shuel, R.W. (1960) Studies on the *in vitro* antitumor activity of fatty acids. I. 10-Hydroxy-2-decenoic acid from royal jelly. *Cancer Res.*, **20**, 503-510.

[57] Kimura, Y. and Atta ur, R. In *Studies in Natural Products Chemistry*; Elsevier, **2008**; Vol. Volume 34, pp. 35-76.

[58] Bincoletto, C.; Eberlin, S.; Figueiredo, C.A.; Luengo, M.B. and Queiroz, M.L. (2005) Effects produced by Royal Jelly on haematopoiesis: relation with host resistance against Ehrlich ascites tumour challenge. *Int. Immunopharmacol.*, **5**, 679-688.

[59] Oršolić, N.; Knezevic, A.; Šver, L.; Terzić, S.; Hackenberger, B.K. and Bašić, I. (2003) Influence of honey bee products on transplantable murine tumours. *Vet Comp. Oncol.*, **1**, 216-226.

[60] Oršolić, N.; Terzić, S.; Šver, L. and Bašić, I. (2005) Honey-bee products in prevention and/or therapy of murine transplantable tumours. *J. Sci. Food Agric.*, **85**, 363-370.

[61] Izuta, H.; Chikaraishi, Y.; Shimazawa, M.; Mishima, S. and Hara, H. (2009) 10-Hydroxy-2-decenoic acid, a major fatty acid from royal jelly, inhibits VEGF-induced angiogenesis in human umbilical vein endothelial cells. *Evid. based Complement. Alternat. Med.*, **6**, 489-494.

[62] Koya-Miyata, S.; Takei, Y.; Ushio, S.; Iwaki, K.; Ikeda, M. and Kurimoto, M. (2002) Royal Jelly and Ascorbic Acid 2-O-α-glucoside (AA-2G) Increase Collagen Synthesis in Normal Hamster Skin Fibroblast Cultures. *Nat.Med.*, **56**, 191-194.

[63] Koya-Miyata, S.; Okamoto, I.; Ushio, S.; Iwaki, K.; Ikeda, M. and Kurimoto, M. (2004), Identification of a collagen production-promoting factor from an extract of royal jelly and its possible mechanism. *Biosci. Biotechnol. Biochem.*, **68**, 767-773.

[64] Spannhoff, A.; Kim, Y.K.; Raynal, N.J.; Gharibyan, V.; Su, M.B.; Zhou, Y.Y.; Li, J.; Castellano, S.; Sbardella, G.; Issa, J.P. and Bedford, M.T. (2011) Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. *EMBO Rep.*, **12**, 238-243.

[65] Fenton, R.G.; Hixon, J.A.; Wright, P.W.; Brooks, A.D. and Sayers, T.J. (1998), Inhibition of Fas (CD95) expression and Fas-mediated apoptosis by oncogenic Ras. *Cancer Res.*, **58**, 3391-3400.

[66] Peli, J.; Schroter, M.; Rudaz, C.; Hahne, M.; Meyer, C.; Reichmann, E. and Tschopp, J. (1999), Oncogenic Ras inhibits Fas ligand-mediated apoptosis by downregulating the expression of Fas. *EMBO J.*, **18**, 1824-1831.

[67] Dhaka, A.; Viswanath, V. and Patapoutian, A. (2006) Trp ion channels and temperature sensation. *Annu. Rev. Neurosci.*, **29**, 135-161.

[68] Caterina, M.J.; Schumacher, M.A.; Tominaga, M.; Rosen, T.A.; Levine, J.D. and Julius, D. (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*, **389**, 816-824.

[69] Terada, Y.; Narukawa, M. and Watanabe, T. (2011) Specific hydroxy fatty acids in royal jelly activate TRPA1. *J. Agric. Food Chem.*, **59**, 2627-2635.

[70] Furukawa, S. (2008) Stimulatory Effects of Royal Jelly on the Generation of Neuronal and Glial Cells - Expectation of Protection Against Some Neurological Disorders -. *Foods Food Ingredients J. Japan,* **213**, 620-626.

[71] Hashimoto, M.; Kanda, M.; Ikeno, K.; Hayashi, Y.; Nakamura, T.; Ogawa, Y.; Fukumitsu, H.; Nomoto, H. and Furukawa, S. (2005) Oral administration of royal jelly facilitates mRNA expression of glial cell line-derived neurotrophic factor and neurofilament H in the hippocampus of the adult mouse brain. *Biosci. Biotechnol. Biochem.*, **69**, 800-805.

[72] Hattori, N.; Nomoto, H.; Fukumitsu, H.; Mishima, S. and Furukawa, S. (2007) Royal jelly and its unique fatty acid, 10-hydroxy-*trans*-2-decenoic acid, promote neurogenesis by neural stem/progenitor cells *in vitro*. *Biomed. Res.*, **28**, 261-266.

[73] Ito, S.; Nitta, Y.; Fukumitsu, H.; Soumiya, H.; Ikeno, K.; Nakamura, T. and Furukawa, S. (2012) Antidepressant-like activity of 10-hydroxy-*trans*-2-decenoic Acid, a unique unsaturated fatty acid of royal jelly, in stress-inducible depression-like mouse model. *Evid. based Complement. Alternat. Med.*, **2012**, ID139140.

[74] Mizukami, Y. (2010) *In vivo* functions of GPR30/GPER-1, a membrane receptor for estrogen: from discovery to functions *in vivo*. *Endocr. J.*, **57**, 101-107.

[75] Prossnitz, E.R. and Maggiolini, M. (2009) Mechanisms of estrogen signaling and gene expression *via* GPR30. *Mol. Cell. Endocrinol.*, **308**, 32-38.

[76] Talukdar, S.; Olefsky, J.M. and Osborn, O. (2011) Targeting GPR120 and other fatty acid-sensing GPCRs ameliorates insulin resistance and inflammatory diseases. *Trends Pharmacol. Sci.*, **32**, 543-550.

[77] Hirasawa, A.; Hara, T.; Katsuma, S.; Adachi, T. and Tsujimoto, G. (2008) Free fatty acid receptors and drug discovery. *Biol. Pharm. Bull.*, **31**, 1847-1851.

[78] Briscoe, C.P.; Tadayyon, M.; Andrews, J.L.; Benson, W.G.; Chambers, J.K.; Eilert, M.M.; Ellis, C.; Elshourbagy, N.A.; Goetz, A.S.; Minnick, D.T.; Murdock, P.R.; Sauls, H.R., Jr.; Shabon, U.; Spinage, L.D.; Strum, J.C.; Szekeres, P.G.; Tan, K.B.; Way, J.M.; Ignar, D.M.; Wilson, S. and Muir, A.I. (2003) The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J. Biol. Chem.*, **278**, 11303-11311.

[79] Hirasawa, A.; Tsumaya, K.; Awaji, T.; Katsuma, S.; Adachi, T.; Yamada, M.; Sugimoto, Y.; Miyazaki, S. and Tsujimoto, G. (2005) Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat. Med.*, **11**, 90-94.

[80] Chu, Z.L.; Carroll, C.; Chen, R.; Alfonso, J.; Gutierrez, V.; He, H.; Lucman, A.; Xing, C.; Sebring, K.; Zhou, J.; Wagner, B.; Unett, D.; Jones, R.M.; Behan, D.P. and Leonard, J. (2010) N-oleoyldopamine enhances glucose homeostasis through the activation of GPR119. *Mol. Endocrinol.*, **24**, 161-170.

[81] Wang, J.; Wu, X.; Simonavicius, N.; Tian, H. and Ling, L. (2006) Medium-chain fatty acids as ligands for orphan G protein-coupled receptor GPR84. *J. Biol. Chem.*, **281**, 34457-34464.

[82] Brown, A.J.; Goldsworthy, S.M.; Barnes, A.A.; Eilert, M.M.; Tcheang, L.; Daniels, D.; Muir, A.I.; Wigglesworth, M.J.; Kinghorn, I.; Fraser, N.J.; Pike, N.B.; Strum, J.C.; Steplewski, K.M.; Murdock, P.R.; Holder, J.C.; Marshall, F.H.; Szekeres, P.G.; Wilson, S.; Ignar, D.M.; Foord, S.M.; Wise, A. and Dowell, S.J. (2003) The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.*, **278**, 11312-11319.

[83] Oh, D.Y.; Talukdar, S.; Bae, E.J.; Imamura, T.; Morinaga, H.; Fan, W.; Li, P.; Lu, W.J.; Watkins, S.M. and Olefsky, J.M. (2010) GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell*, **142**, 687-698.

[84] Hirakawa, A.; Shimizu, K.; Fukumitsu, H.; Soumiya, H.; Iinuma, M. and Furukawa, S. (2010) 2-Decenoic acid ethyl ester, a derivative of unsaturated medium-chain fatty acids, facilitates functional recovery of locomotor activity after spinal cord injury. *Neuroscience*, **171**, 1377-1385.

[85] Tanaka, Y.; Fukumitsu, H.; Soumiya, H.; Yoshimura, S.; Iwama, T. and Furukawa, S. (2012) 2-Decenoic Acid Ethyl Ester, a Compound That Elicits Neurotrophin-like Intracellular Signals, Facilitating Functional Recovery from Cerebral Infarction in Mice. *Int. J. Mol. Sci.*, **13**, 4968-4981.

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