療法，化学療法に従事する医師などの育成に加え，手術療法の充実ならびに専門的に行う医療従事者の育成が盛り込まれました。外科を専攻する医師などの減少の危惧から新たに重点化されたものです。また，対象が医療従事者全体へと拡がったのも注目すべきところです。

さらに，②緩和ケアの実施が「治療の初期段階からの」という文言から，よりわかりやすい「がんと診断された時からの」という文言に変わりました。

③がん登録の推進について，第一次計画と同様に重点項目として盛り込まれました。がん登録については，第一次計画でほとんど達成されていなかった項目です。がん登録促進のためのさまざまな工夫，提言も第二次計画には挙げられています(後述)。

新しく重点項目となったものとして，④働く世代のがん患者に対する就労問題などの対策，また第一次計画で抜け落ちていた小児がんに対するがん対策の充実が加わりました。

「全体目標」については，①がんによる死亡者の20％減は第一次計画と同様の目標です。これまでの５年間で8.8％の減少であったので，20％を達成するためには今後の５年で11.2％減少を達成する必要があります。②「全てのがん患者とその家族の苦痛の軽減と療養生活の質の維持向上」については，目標の文言について変化はありません。新たに加わったのが，③「がんになっても安心して暮らせる社会の構築」です。がん体験者の意見・体験をもとに新たに加えられた重要な項目です。

また，第二次計画のなかの「分野別施策と個別目標」についても，第一次計画の反省，加えてがん体験者の視点をもとに，さまざまな個別項目が第一次計画から内容が進化しています。また，第一次計画には盛り込まれていなかった内容についても，前述の「小児がん」をはじめ「医薬品・医療機器の早期開発・承認等に向けた取組」「がんの教育・普及啓発」「がん患者の就労を含めた社会的な問題」など，さまざまな提言が新たに加わりました。すべてをここで紹介することはできませんが，特にがん患者，がん体験者の視点をもとに盛り込まれた内容について主立ったものを以下に紹介させていただきます。

がん体験者の視点による提言

「第二次がん対策推進基本計画」では，新たに重点的に取り組む課題として「働く世代や小児へのがん対策の充実」，そして全体目標として「がんになっても安心して暮らせる社会の構築」が盛り込まれました。

これは，がん対策推進協議会委員であるがん体験者およびその家族，さらにはアドバイザーとしての多くのがん体験者による，がん対策推進協議会をはじめとしたさまざまな委員会での意見が取り上げられ盛り込まれたものであると思われます。

「働く世代や小児へのがん対策の充実」での，働く世代へのがん対策，特にがん患者の就労問題への対策は，まさにがん体験者の当面している問題です。

がんは，もちろん高齢者に多く発症するものですが，がん患者の30％は働く世代である20～64歳にみられます。今般の手術療法，放射線療法，化学療法の進歩によりがん患者の生存率はかなり上がり，抗がん剤治療を受けながら働いている患者も増えてきています。今回の計画には，特にがん患者やがん体験者の就労について，患者の経済的負担を軽くすることや，働いている企業などにも協力を求め働きながら治療を受けることができる仕組みを作ることなどが盛り込まれました。

多くの数値目標の導入

第二次がん対策推進基本計画には多くの数値目

標が導入され，具体的な結果を求められる計画になっています。

1．喫煙率の削減

分野別施策と個別目標の「がんの予防」において，喫煙率の抑制についての数値目標が導入されました。10年後(2022年)までに，現状の19.5%の喫煙率を12.2%に引き下げるものです。この数字は，19.5%の喫煙者のうちアンケート調査などでたばこを止めたいと思っている喫煙者の数(約40%)を喫煙ゼロとした場合の数字であるとのことです。喫煙率は第一次がん対策推進基本計画でもたたき台の段階では「半減させる」と盛り込まれる予定であったものが見送られた事実があり，今回はかなり踏み込んだものと考えられます。また，受動喫煙の減少についても数値目標が盛り込まれました。

2．がん検診の受診率の数値目標導入

分野別施策と個別目標の「がんの早期発見」において，がん検診の受診率向上に向けてきめ細かな数値目標が導入されました。第一次がん対策推進基本計画でも数値目標は設定されていたものの，5年の実践のなかでそれは全く数値に届いておらず，非現実的な数値目標といえるものであったという反省からのきめ細かな数値目標の導入です。

・受診率算定の対象を，国際基準にあわせて40～69歳(子宮頸がんのみ20～69歳)とする。
・子宮頸がん，乳がんの検診を50%以上とする。
・胃，肺，大腸がんは当面40%をめざす。

一方，がん検診そのものが患者のために本当に有効であるかなどについてもしっかりと議論すべきであるという点も盛り込まれました。

3．がん登録の数値目標

分野別施策と個別目標の「がん登録」において，平成24年度中に47都道府県すべてでがん登録事業を行うこと，また5年以内にがん患者にがん登録と予後調査を行うなどがん登録の精度を上げること，加えてがん登録の法的位置づけの検討を行うことが盛り込まれました。

おわりに

がん対策を総合的に推進するためには，関係者の連携・協力を進めることのできる体制整備や，都道府県による計画策定を充実させること，またがん対策関係者の意見を集約し把握できる体制を整備すること，さらにがん登録などにおける国民の協力も不可欠です。そのための措置をしっかりと行うことも計画に盛り込まれています。

このように，第一次計画と比べて第二次計画はかなり実践的，そして前衛的なものになっていますが，まだまだ議論が尽くされていない，進め方がはっきりとしていない点も見受けられます。

緩和ケアの質の評価については確たる評価法が定まっていません。たとえば，がんの痛みがどのように取れたかという「除痛率」などを指標として用いるべきであること。

ドラッグ・ラグ問題については，開発者，認可する機関，がん患者などが一同に会して検討しあう場所を設置し包括的に議論すべきであること。

特に適応外薬の問題では，緩和医療での痛み対策に使われる薬剤に適応外薬が多いことから，その解決をめざすべきであること。

などが，がん対策推進協議会委員からも指摘されています。

まだ改善する部分もあるとはいえ，第一次計画からはかなりの進歩，進捗のみられる計画であることは異論のないところです。

第二次がん対策推進基本計画の閣議決定後，同計画に則ってがん対策をしっかりと進めていくことが一番の近道であるので，その対応を見守っていきたいと思います。

追い風となる報告として，以下の記事を紹介し

て本稿を締めくくりたいと思います。

2012年３月13日，日本医学会，日本癌学会，日本癌治療学会，日本臨床腫瘍学会の４学会より，「がん登録の法制化を！」という要望書が小宮山洋子厚生労働大臣に出されました[7]。がん登録は，第一次，第二次がん対策推進基本計画ともに重点課題として挙げられているものです。平成24年度中には全国47都道府県のすべてで地域がん登録および院内がん登録事業が行われることとなりましたが，各地域での登録の現状には温度差があり，いまだ古いデータを用いているところもあります。がん登録ががんの現状を把握するために必須であるにもかかわらずなかなか普及しないのは，登録情報を今後どのように用いるか，どのようにがん患者にその情報が還元されるのかといった道筋がはっきりしていないことにあると思われます。全国むらなくデータを集め，きめ細かい正確なデータを集めることが最重要であることを鑑み，前述の４団体はがん登録事業を国の事業として位置づけ，100％の登録率をめざすことを提言しています。国によるがん登録事業の法制化が整備されれば，飛躍的に登録事業が進むことが期待されます。

また，2012年３月16日の厚生労働省プレスリリースによると，４月１日よりこれまでがん対策政策を担当していた健康局総務課設置のがん対策推進室が発展的に拡大され，生活習慣病対策室，地域保健室，保健指導室を再編し「がん対策・健康増進課」になることが決まりました[6]。室から課へと昇格したことで，さらに各室と連携した相互的政策，がん対策が強力に推進できる体制となるであろうと期待されます。

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Short Communication

Inhibition by Pregnenolone Sulphate, a Metabolite of the Neurosteroid Pregnenolone, of Voltage-Gated Sodium Channels Expressed in *Xenopus* Oocytes

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Abstract. Neurosteroids are known as allosteric modulators of the ligand-gated ion channel superfamily. Voltage-gated sodium channels (Na_v) play an important role in mediating excitotoxic damages. Here we report the effects of neurosteroids on the function of Na_v, using voltage-clamp techniques in *Xenopus* oocytes expressed with the $Na_v1.2$ α subunit. Pregnenolone sulphate, but not pregnenolone, inhibited sodium currents (I_{Na}) at 3 – 100 μmol/L. The suppression of I_{Na} by pregnenolone sulphate was due to increased inactivation with little change in activation. These findings suggest that pregnenolone sulphate, a metabolite of pregnenolone, suppresses the function of Na_v via increased inactivation, which may contribute to the neuroprotection.

Keywords: pregnenolone sulphate, voltage-gated sodium channel, neuroprotection

Neurosteroids are active steroids synthesized in both the central and peripheral nervous systems, independent of a steroidogenic gland. It is well known that neurosteroids modify the activity of neurotransmitter-gated ion channels and rapidly alter neuronal excitability. Several lines of evidence have shown that these neurosteroids modulate excitotoxicity-mediated neuronal damage through glutamate receptors. For example, pregnenolone and dehydroepiandrosterone (DHEA) protect hippocampal neurons against NMDA-induced neuronal death (1), and pregnenolone sulphate attenuates AMPA-induced inward currents and neurotoxicity in cortical neurons (2). These results suggest neuroprotective effects of neurosteroids and suggest their potential use as a therapeutic medication for brain damage. However, the entire signal transduction cascade of neurosteroids on excitotoxic neuronal injury is still obscure.

Voltage-gated sodium channels play an essential role in action potential initiation and propagation in excitable cells of nerves and muscles (3, 4). Recently, sodium channels are thought to have an important role in excitotoxic damage because sodium channel blockers have neuroprotective effects (5). Here, we examined the effects of neurosteroids on voltage-gated sodium channels using the $Na_v1.2$ α subunit of sodium channels because it is expressed primarily in the central nervous system, especially the brain. We found that pregnenolone sulphate, but not pregnenolone, inhibited the function of $Na_v1.2$ expressed in *Xenopus* oocytes.

Adult female *Xenopus laevis* frogs were obtained from Kyudo Co., Ltd. (Saga). Pregnenolone, pregnenolone sulphate, DHEA, and DHEA sulphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). cDNA for rat $Na_v1.2$ α subunits was a gift from Dr. W.A. Catterall (University of Washington, Seattle, WA, USA), and cDNA for human β_1 subunits was a gift from Dr. A.L. George (Vanderbilt University, Nashville, TN, USA). After linearized cDNA, cRNA were transcribed using T7 ($Na_v1.2$ α subunits) or T6 (β_1 subunits) RNA polymerase of mMESSAGE mMACHINE kit (Ambion, Austin, TX,

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USA). Preparation of *Xenopus laevis* oocytes and microinjection of the cRNA was performed as described previously (6). $Na_v1.2$ α subunits cRNA was co-injected with β_1 subunits cRNA into oocytes. All electrical recording was performed at room temperature (23°C) using the whole-cell, two-electrode, voltage-clamp technique. The oocytes were perfused at 2 mL/min with Frog Ringer solution containing 115 mmol/L NaCl, 2.5 mmol/L KCl, 10 mmol/L HEPES, and 1.8 mmol/L $CaCl_2$, at pH 7.2. The whole-cell voltage clamp was achieved through these two electrodes using a Warner Instruments model OC-725C (Warner, Hamden, CT, USA). Currents were recorded and analyzed using pCLAMP software (Axon Instruments, Foster City, CA, USA). Neurosteroids were perfused for 2 min to reach equilibrium. All values are presented as the mean ± S.E.M. Data was statistically evaluated by paired *t*-test using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, USA).

First, currents were elicited using a 50-ms depolarizing pulse of −20 mV applied every 10 s from a −90 mV holding potential (causing maximal current, $V_{H\text{-}90}$) or a holding potential causing half-maximal current ($V_{1/2}$) (approximately from −50 to −60 mV) in the absence and presence of neurosteroids. The peak current amplitude in presence of neurosteroids was normalized to that in the control, and the effects of neurosteroids were expressed as percentages of the control. Pregnenolone (100 μmol/L) had little effect on sodium currents (I_{Na}) at both $V_{H\text{-}90}$ and $V_{1/2}$ (Fig. 1: A, B, left). Pregnenolone sulphate (100 μmol/L) significantly suppressed I_{Na} to 41% ± 3% of the control at $V_{1/2}$ and to 74% ± 3% at $V_{H\text{-}90}$ (Fig. 1: A, B, right). In addition to pregnenolone sulphate, DHEA and DHEA sulphate (100 μmol/L) also reduced I_{Na} to 50% ± 2% and 40% ± 4% at $V_{1/2}$ and to 69% ± 3% and 74% ± 3% at $V_{H\text{-}90}$, respectively (Fig. 1C). Next, we examined the concentration–response relation for inhibition of I_{Na} by neurosteroids at the $V_{1/2}$ (Fig. 2). Pregnenolone sulphate (3 – 300 μmol/L) significantly attenuated I_{Na} in a concentration-dependent manner. The effects of DHEA and DHEA sulphate were 10-fold less potent than that of pregnenolone sulphate. Only ≥ 30 μmol/L of DHEA and DHEA sulphate significantly suppressed I_{Na}. Nonlinear regression analyses of the concentration–response curves yielded the IC_{50} values and Hill slopes of 53 ± 1 and 0.5 ± 0.05 μmol/L for pregnenolone sulphate, 130 ± 8 and 0.8 ± 0.04 μmol/L for DHEA, and 202 ± 6 and 0.9 ± 0.09 μmol/L for DHEA sulphate, respectively.

Since pregnenolone sulphate most potently affected

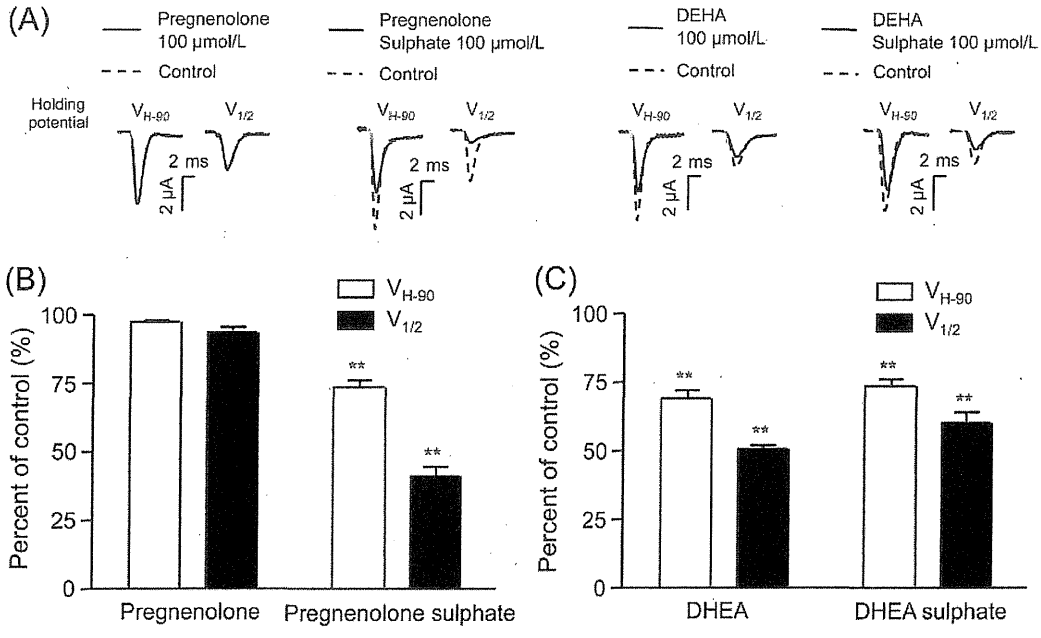

Fig. 1. Effects of pregnenolone, pregnenolone sulphate, DHEA, and DHEA sulphate on sodium currents in *Xenopus* oocytes expressing $Na_v1.2$ α subunits with β_1 subunits. A) Representative traces of sodium currents evoked by 50-ms depolarizing pulses to −20 mV from a $V_{H\text{-}90}$ holding potential and $V_{1/2}$ holding potential, in the absence and presence of neurosteroids. Each neurosteroid of at 100 μmol/L was applied for 2 min. B, C) The inhibition of sodium currents by pregnenolone, pregnenolone sulphate, DHEA, and DHEA sulphate. The currents were normalized to the initial values, and percent of the control was calculated. Open columns indicate the effect at $V_{H\text{-}90}$, and closed columns indicate the effect at $V_{1/2}$. Data are represented as means ± S.E.M. (n = 5 – 6). $^{**}P < 0.01$, compared with the control (paired *t*-test).

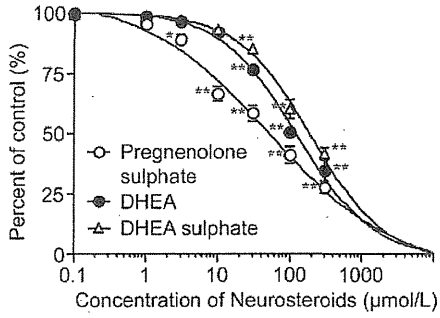

Fig. 2. Concentration–response curves for pregnenolone sulphate, DHEA, and DHEA sulphate of suppression of sodium currents. Sodium currents were elicited by a 50-ms depolarizing pulse to −20 mV from a $V_{1/2}$ holding potential. Data are represented as means ± S.E.M. (n = 5). The data were fit by the Hill equation to give the IC_{50} values and Hill slopes. IC_{50} values and Hill slopes were calculated by GraphPad Prism. $^*P < 0.05$ and $^{**}P < 0.01$ (paired *t*-test).

the function of $Na_v1.2$, to investigate the inhibitory mechanism of this steroid, we studied the effect of it on sodium current activation and steady-state inactivation. The voltage dependence of activation was determined using 50-ms depolarizing pulses from V_{H-90} and $V_{1/2}$ to 60 mV in 10-mV increments. Normalized activation curves were fitted to a Boltzmann equation (6). The peak I_{Na} was reduced by 100 μmol/L of pregnenolone sulphate at V_{H-90} and $V_{1/2}$ (not shown), but it did not affect the activation curves at V_{H-90} and $V_{1/2}$ (Fig. 3: A, B). Next, currents were elicited by a 50-ms test pulse to −20 mV after 200-ms prepulses ranging from −140 to 0 mV in 10-mV increments from V_{H-90}. Steady-state inactivation curves were fitted to the Boltzmann equation (6). Pregnenolone sulphate shifted the $V_{1/2}$ in the hyperpolarizing direction significantly by 8.2 mV (from −53 ± 1 to −61.3 ± 1 mV) (Fig. 3C). To the best of our knowledge, this is the first direct evidence to demonstrate the inhibitory effect of pregnenolone sulphate on the function of sodium chan-

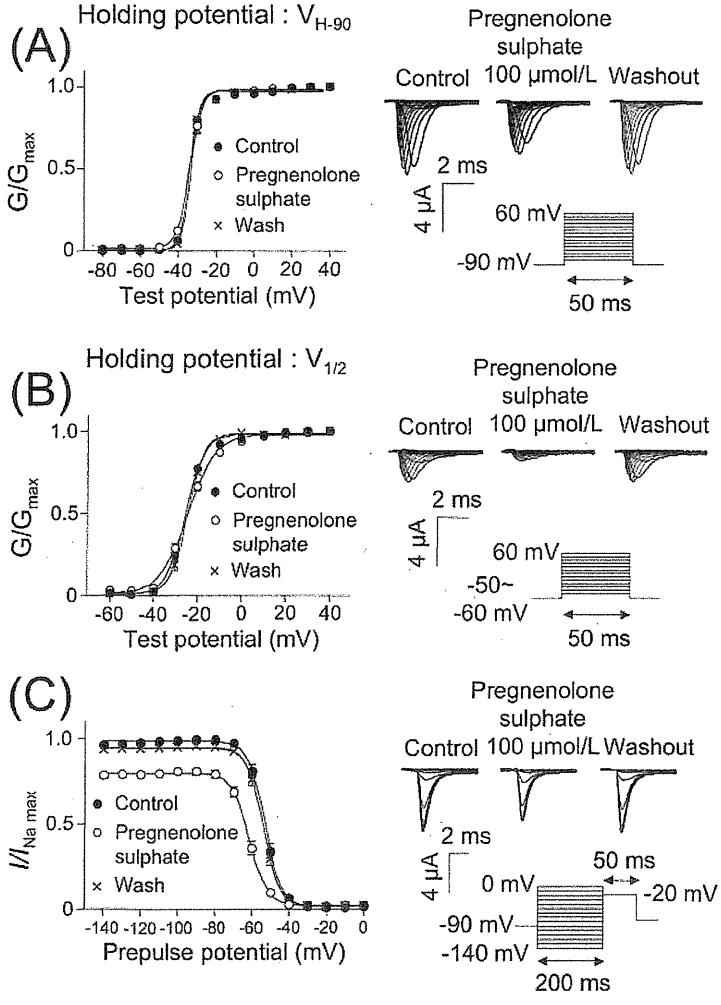

Fig. 3. Effects of pregnenolone sulphate on activation curves at V_{H-90} holding potential (A) and $V_{1/2}$ holding potential (B) and on inactivation curves (C). Currents were elicited using 50-ms depolarizing steps between −80 and 60 mV in 10-mV increments from a V_{H-90} holding potential (A) and elicited using 50-ms depolarizing steps between −60 and 60 mV in 10-mV increments from a $V_{1/2}$ holding potential (−50 to −60 mV) (B). Pregnenolone sulphate (100 μmol/L) were applied for 2 min. Representative I_{Na} traces from oocytes expressing $Na_v1.2$ with β_1 in the control, presence of 100 μmol/L pregnenolone sulphate, and washout (right panel). Activation curves were fitted to a Boltzmann equation from the I-V curves (left panel). Closed circles represent the control, open circles indicate the effects of pregnenolone sulphate, and crosses indicate washout. Data are shown as the mean ± S.E.M. (n = 5 – 6). C) Currents were elicited using a 50-ms test pulse to −20 mV after 200-ms prepulses ranging from −140 to 0 mV in 10-mV increments from a holding potential of V_{H-90}. Pregnenolone sulphate was applied for 2 min. Representative I_{Na} traces in the control, presence of 100 μmol/L pregnenolone sulphate, and washout (right panel). Inactivation curves were fitted to the Boltzmann equation (left panel). Closed circles represent the control, open circles indicate the effects of pregnenolone sulphate, and crosses indicate washout. Data are shown as the mean ± S.E.M. (n = 5 – 7).

nels of $Na_v1.2$ via increased inactivation.

We found that pregnenolone sulphate, DHEA, and DHEA sulphate, but not pregnenolone, suppressed I_{Na} of $Na_v1.2$ α subunits. Specifically, pregnenolone sulphate had the most potent inhibitory effect among them. Typical plasma concentrations of pregnenolone sulphate have been reported to be 0.2 to 0.4 μmol/L, although those in some healthy subjects have been reported to be approximately 1 μmol/L (7), and normal serum levels of DHEA and DHEA sulphate have been shown to be 10 – 30 nmol/L and 2.0 – 3.0 μmol/L, respectively (8). In some cases and conditions, during pregnancy and in patients with 21-hydroxylase deficiency, however, plasma levels of these neurosteroids can reach micromolar levels (9, 10). The present findings showed that 3 μmol/L pregnenolone sulphate significantly suppressed I_{Na} to 89% ± 2% of the control value. Scholz et al. showed that 10% inhibition of I_{Na} by lidocaine reduced the number of action potentials to 10 from a control response of 21 in 750 ms (11). Therefore, 11% inhibition by 3 μmol/L pregnenolone sulphate would be expected to reduce action potentials. In contrast to pregnenolone sulphate, DHEA and DHEA sulphate suppressed I_{Na} at only high concentrations ($\geq$ 30 μmol/L). In consideration of the above observations, only pregnenolone sulphate would affect neuronal functions through sodium channel inhibition under pathophysiological conditions. In a previous study, pregnenolone as well as pregnenolone sulphate affected the ligand-gated ion channel, $GABA_A$ receptor (12). Therefore, it is interesting to note that only the sulphated steroid of pregnenolone, but not non-sulphated pregnenolone, has an inhibitory effect on sodium channels, suggesting that sulphotransferase and the sulphate moiety of pregnenolone sulphate might play an important role in regulating sodium channel activity. Contrary to pregnenolone and pregnenolone sulphate, both DHEA and DHEA sulphate had similar effects, suggesting that the important moiety in DHEA and DHEA sulphate would be different from that of pregnenolone sulphate.

The present results of channel gating such as activation and inactivation suggest that the enhanced inactivation of sodium channels with little change in activation contributes to the inhibitory mechanism of pregnenolone sulphate. Furthermore, suppression by pregnenolone sulphate at V_{H-90} suggests that it behaves as open channel blockers. This mechanism resembles that of the local anesthetic lidocaine in the point of causing a hyperpolarizing shift in the voltage dependence of steady state inactivation with no effect on that of activation. These results have led us to hypothesize that pregnenolone sulphate and lidocaine, hydrophobic tertiary amine local anesthetics, might share common or overlapping binding sites.

Suppression of voltage-gated sodium channels would be supposed to have a neuroprotective effect because the inhibition of sodium channels leads to suppression of neuronal depolarization, glutamate release, and Na^+ influx, thereby reducing Ca^{2+} influx by means of Ca^{2+} channels, NMDA receptors, and reversal of the Na^+/Ca^{2+} exchanger (13). Pregnenolone sulphate also has effects on the activities of some ion channels, for example, the subtype selective modulation of NMDA receptor (14) and antagonism of the $GABA_A$ receptor and suppression of GABA-induced current (15) at micromolar concentrations. However, it is not clear whether the effects of these modulations of the other receptors can lead to neuronal damage, whereas some new sodium-channel blockers have been shown to have neuroprotective effects (5). Therefore, it gives rise to the possibility that pregnenolone sulphate may be used as a potential neuroprotective medication for the treatment of brain injury. Further in vivo study, however, will be required in the near future.

In conclusion, the present findings demonstrated that pregnenolone sulphate suppresses I_{Na} of $Na_v1.2$ α subunits. These findings would provide a better understanding of the mechanisms underlying the neuroprotective effects exerted by neurosteroids.

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Sensation of Abdominal Pain Induced by Peritoneal Carcinomatosis Is Accompanied by Changes in the Expression of Substance P and μ-Opioid Receptors in the Spinal Cord of Mice

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ABSTRACT

Background: Patients with peritoneal carcinomatosis often report abdominal pain, which is relatively refractory to morphine. It has been considered that a new animal model is required to investigate the mechanism of abdominal pain for the development of optimal treatments for this type of pain.

Methods: To prepare a peritoneal carcinomatosis model, highly peritoneal-seeding gastric cancer cells, 60As6, were implanted into the abdominal cavity. The nociceptive modality for pain-related behavior was assessed in terms of withdrawal behavior in response to mechanical stimuli and hunching behavior. Tissue samples from mouse dorsal root ganglia and spinal cord were subject to immunohistochemistry and real-time reverse transcription polymerase chain reaction.

Results: Mice with peritoneal dissemination showed significant hypersensitivity of the abdomen to mechanical stimulation and spontaneous visceral pain-related behavior. There was a significant increase in c-Fos–positive cells in the spinal cord in tumor-bearing mice. Those mice exhibited a remarkable increase in substance P-positive neurons in the dorsal root ganglia (control *vs.* tumor, 15.4 ± 1.1 *vs.* 24.2 ± 3.6, $P < 0.05$, n = 3). A significant decreases in μ-opioid receptor expression mainly in substance P-positive neurons was observed in tumor-bearing mice (69.3 ± 4.9 *vs.* 38.7 ± 0.9, $P < 0.05$, n = 3), and a relatively higher dose of morphine was required to significantly reverse the abdominal hypersensitivity.

What We Already Know about This Topic

- Some patients with cancer have tumor spread throughout the abdominal peritoneum
- The disease of widespread peritoneal tumors, carcinomatosis, can be painful and refractory to conventional analgesic therapies

What This Article Tells Us That Is New

- The investigators developed a mouse model of carcinomatosis
- Behavioral studies indicate pain-related responses are present, and as in patients, the responses are resistant to morphine
- In the future, experimental pain therapies can be studied

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Conclusion: Both the up-regulation of substance P and down-regulation of μ-opioid receptor seen in the dorsal root ganglia may be, at least in part, responsible for the abdominal pain-like state associated with peritoneal carcinomatosis.

THE moderate or severe pain associated with cancer can impair dramatically the quality of life and the survival of patients and affects 64% of those with metastatic or advanced-stage cancer.[1] The perceived intensity of this pain depends on the specific type of cancer and its location, as well as the each patient's sensitivity to pain.[2] Pain associated with cancer generally is treated with opioids, nonsteroidal antiinflammatory drugs, corticosteroids, local anesthetics, antidepressants, and anticonvulsants, either alone or in combination.[3,4] Despite the availability of these various medicinal treatments, it can be difficult to control pain in some patients with terminal cancer. Accordingly, more effective treatments are needed. Our poor understanding of the mechanism of pain associated with cancers continues to stand as a major obstacle to the discovery of novel analgesics to address this need.

Peritoneal carcinomatosis has been defined as the complex sequence of events by which tumor cells disseminate from their primary organ of origin to establish independent metastatic deposits on the visceral and parietal peritoneal lining of the abdominal cavity. Advanced gastric cancer with peritoneal dissemination is one of the most difficult forms of gastric cancer to treat, and its prognosis remains poor.[5] Patients with peritoneal carcinomatosis report constant, aching abdominal pain. Characteristically, it is poorly localized and is worsened by pressure on the abdomen. Although there have been no rigorous clinical studies, most experts agree that opiate analgesics are relatively ineffective for the treatment of abdominal pain caused by cancerous peritonitis. Compared with somatic pain, which is easily localized and characterized by distinct sensations, visceral pain is diffuse and poorly localized, typically referred to somatic sites, and associated with stronger emotional and autonomic reactions.[6] The abdominal viscera receive dual extrinsic innervation (*e.g.*, spinal and vagal afferents), and accumulating evidence has revealed that there are significant differences in the functions of different nerves innervating the same organ.[6] Adequate stimuli for the production of visceral pain include the distension of hollow organs, traction on the mesentery, ischemia, and endogenous chemicals typically associated with inflammatory processes.[6] Thus, visceral pain differs from somatic pain in several important ways. Several new animal models have been developed for the investigation of cancer pain.[7] The first animal models were developed for the study of primary and metastatic bone tumors,[8] and these were followed by nonbone models of cancer pain that resemble other malignant lesions.[9,10] These animal models were established to enhance our understanding of the neurobiology, pharmacology, and molecular mechanisms of tumor pain.[11–14] However, these are mostly models for somatic pain. The development of optimal analgesic medications for the treatment of abdominal pain caused by cancerous peritonitis has been hindered by our incomplete understanding of the underlying mechanisms, mainly because of a lack of appropriate animal models to study. In this study, we used 60As6 gastric cancer cells, which are a highly peritoneal-seeding cell line, to develop a novel mouse model of abdominal pain caused by cancer-related peritonitis. Information obtained with this model has provided new insight into the mechanisms that underlie pain caused by cancerous peritonitis and may aid the establishment of potential mechanism-based therapies for treating this pain state.

Materials and Methods

All experiments were conducted in accordance with the ethical guidelines of the International Association for the Study of Pain[15] and were approved by the Committee for Ethics of Animal Experimentation of National Cancer Center (Tsukiji, Tokyo, Japan). In the experiments, efforts were made to minimize the numbers of animals used and their suffering.

Animals

Male C.B17/Icr-scid mice and Institute of Cancer Research mice weighing 22–25 g were used. Mice were purchased from CLEA Japan (Tokyo, Japan) and housed at a room temperature of 23 ± 1°C with a 12-h light–dark cycle. The mice were maintained under specific pathogen-free conditions and provided sterile food, water, and cages.

Cell Lines and Culture

A human scirrhous gastric cancer cell line, HSC60, was established as described previously.[16] The highly peritoneal-seeding cell line, 60As6 was established from HSC60 by orthotopic tissue implantation into scid mice.[17] Briefly, a xenograft tumor of HSC60 cells was transplanted into the gastric wall of a scid mouse. For six iterations, we harvested ascitic tumor cells and performed the orthotopic inoculation of these cells into mice to establish a highly metastatic 60As6 cell line. This cell line was maintained in RPMI1640 medium supplemented with fetal calf serum (10%), 100 U/ml penicillin G sodium, and 100 μg/ml streptomycin sulfate under an atmosphere of 5% carbon dioxide and 95% air at 37°C. To establish transfectants that expressed the luciferase gene, plasmid vectors carrying the firefly luciferase gene, which were called pLuc/Neo, and a transfection reagent, LipofectAMINE 2000 (Invitrogen, Carlsbad, CA), were used as recommended by the manufacturer. Geneticin (500 μg/ml; Invitrogen) was used to select stable transfectants, and transfected clones were screened for luciferase gene expression by detecting bioluminescence using an IVIS system (Xenogen, Alameda, CA). Clones that expressed the luciferase gene were referred to as 60As6Luc cells.