multiple comparisons. Hill slope, half-maximal inhibitory concentration (IC_{50}), and half-maximal effective concentration (EC_{50}) values were also calculated. *P* value less than 0.05 was considered to indicate a significant difference.

Results

Effects of APAS and PAS on Peak Na+ Inward Currents Elicited from Two Different Holding Potentials

Currents were elicited using a 50-ms depolarizing pulse to −20 mV for $Na_v1.2$ and $Na_v1.6$, −10 mV for $Na_v1.7$, and +10 mV for $Na_v1.8$ applied every 10 s from a V_{max} or $V_{1/2}$ holding potential in both the absence and presence of 100 μmol/l APAS and PAS (fig. 2). The amplitude of expressed sodium currents was typically 2 to 15 μA, and oocytes that showed a maximal current greater than 20 μA were not included in the data collection in all the following experiments. APAS had dual effects on sodium currents depending on the holding potential and α subunit (figs. 2 and 3). At $V_{1/2}$, APAS reduced the peak I_{Na} (sodium current) induced by $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$ by 79 ± 1%, 71 ± 2%, and 49 ± 3%, respectively. At V_{max}, APAS also reduced I_{Na} induced by $Na_v1.2$ by 60 ± 4%, whereas it enhanced I_{Na} induced by $Na_v1.6$ and $Na_v1.7$ by 15 ± 6% and 14 ± 1%, respectively, although these effects were small. In contrast, APAS greatly enhanced I_{Na} induced by $Na_v1.8$ at both $V_{1/2}$ and V_{max} by 112 ± 34% and 202 ± 14%, respectively (fig. 3A). PAS reduced I_{Na} induced by $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$ at $V_{1/2}$ by 54 ± 4%, 71 ± 1%, and 48 ± 2%, respectively. Effects of PAS on I_{Na} at V_{max} were smaller than those at $V_{1/2}$, and the magnitudes of inhibitory effects on $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$ were 31 ± 5%, 10 ± 1%, and 6 ± 1%, respectively. While PAS enhanced I_{Na} induced by $Na_v1.8$ at V_{max} by 39 ± 6%, it did not affect I_{Na} induced by $Na_v1.8$ at $V_{1/2}$ (fig. 3B). In summary, PAS inhibited I_{Na} induced by $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$ at both $V_{/1/2}$ and V_{max} holding potentials. APAS had inverse effects on $Na_v1.6$ and $Na_v1.7$ according to the different holding potentials, whereas it suppressed I_{Na} induced by $Na_v1.2$ at both $V_{/1/2}$ and V_{max}. Moreover, APAS markedly enhanced I_{Na} induced by $Na_v1.8$ at both $V_{/1/2}$ and V_{max}.

Next, we examined the concentration–response relationship for suppression of the peak I_{Na} induced through $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$ by APAS and PAS at $V_{1/2}$ holding potential because suppression by both neurosteroids of these α subunits at $V_{1/2}$ was more potent than that at V_{max} (fig. 4, A and B). In addition, we investigated the concentration–response relationship for potentiation of the peak I_{Na} of $Na_v1.8$ by APAS and PAS at V_{max}, because both neurosteroids showed potent enhancement of I_{Na} at V_{max} compared with that at $V_{1/2}$ (fig. 4C). IC_{50} values, EC_{50} values, and Hill slopes calculated from nonlinear regression analyses of the dose–response curves are shown in table 1. From these analyses, the effect of APAS on $Na_v1.2$ was the most potent among the two neurosteroids and four α subunits.

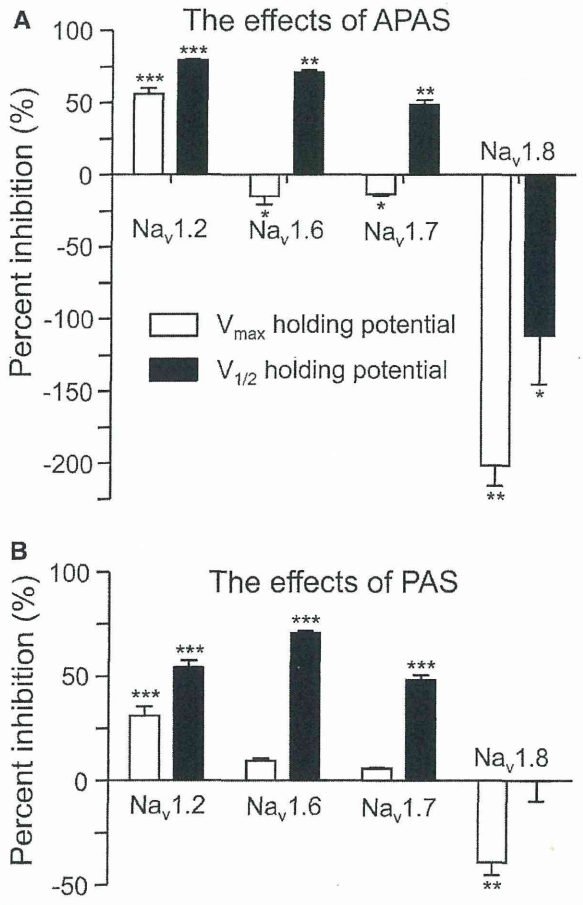

Fig. 3. Percentage inhibition of sodium currents of allopregnanolone sulfate (APAS) (n = 6) (*A*) and pregnanolone sulfate (PAS) (n = 5) (*B*) were calculated. *Open columns* represent the effect at V_{max} holding potential, and *closed columns* indicate the effect at $V_{1/2}$. Data are presented as means ± SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 compared with the control, based on paired *t* test (two-tailed). Na_v = voltage-gated sodium channel; V_{max} holding potential = holding potential causing maximal current; $V_{1/2}$ holding potential = holding potential causing half-maximal current.

Effects of APAS and PAS on Activation of Sodium Currents

We examined the effects of APAS and PAS on four α subunits in sodium current activation. Voltage dependence of activation was determined using 50-ms depolarizing pulses from a holding potential of V_{max} to 50 mV in 10-mV increments or from a holding potential of $V_{1/2}$ to 60 mV in 10-mV increments for $Na_v1.2$, $Na_v1.6$, $Na_v1.7$, and $Na_v1.8$ in both the absence and presence of 100 μmol/l APAS and PAS (fig. 5). Activation curves were derived from the I–V curves (see Electrophysiological Recordings under Materials and Methods). At V_{max}, APAS greatly reduced the peak I_{Na} induced by $Na_v1.2$, whereas it greatly enhanced the peak I_{Na} induced by $Na_v1.8$ in the depolarizing region where channel opening begins. It also enhanced the peak I_{Na} induced by $Na_v1.6$ and $Na_v1.7$, similar to its effects on $Na_v1.8$,

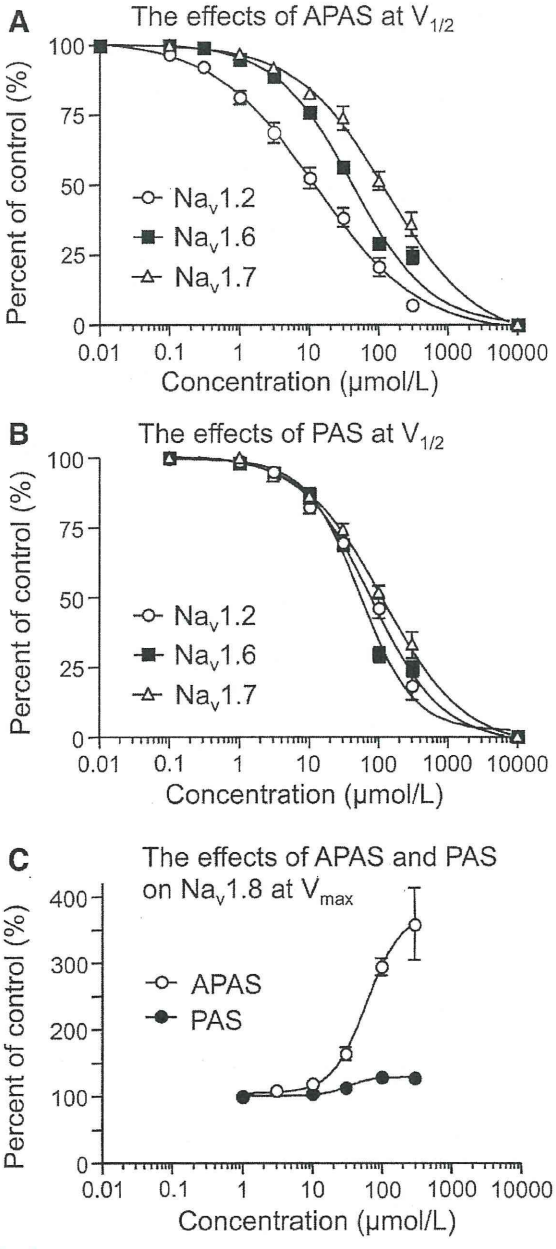

Fig. 4. Concentration–response curves for two-compound suppression of sodium currents elicited by 50-ms depolarizing pulses to −20 mV for $Na_v1.2$ (n = 6) and $Na_v1.6$ (n = 7) and −10 mV for $Na_v1.7$ (n = 5) from $V_{1/2}$ holding potential (*A* and *B*) and those for two-compound potentiation of sodium currents elicited by 50-ms depolarizing pulses to +10 mV for $Na_v1.8$ (n = 5) from V_{max} (*C*). The peak current amplitude in the presence of two compounds was normalized to that of the control, and the effects are expressed as percentages of the control. Hill slopes, IC_{50} values, and EC_{50} values are shown in table 1. Data are presented as means ± SEM. Data were fitted to the Hill slope equation to give the Hill slopes, IC_{50} values, and EC_{50} values. Hill slopes, IC_{50} values, and EC_{50} values were calculated using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). APAS = allopregnanolone sulfate; Na_v = voltage-gated sodium channel; PAS = pregnanolone sulfate; V_{max} = holding potential causing maximal current; $V_{1/2}$ = holding potential causing half-maximal current.

although both effects were small. At $V_{1/2}$, APAS greatly suppressed the peak I_{Na} induced by $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$, but it enhanced the peak I_{Na} induced by $Na_v1.8$, similar to its effects on $Na_v1.8$ at V_{max}. PAS reduced I_{Na} induced by $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$ at both $V_{1/2}$ and V_{max}, whereas it enhanced I_{Na} induced by $Na_v1.8$ in the depolarizing region at V_{max}, but had no effect at $V_{1/2}$.

At V_{max} holding potential, APAS significantly shifted the midpoint of the steady-state activation ($V_{1/2}$) in a depolarizing direction for $Na_v1.2$, but it significantly shifted $V_{1/2}$ in a hyperpolarizing direction for $Na_v1.6$, $Na_v1.7$, and $Na_v1.8$. At $V_{1/2}$, APAS also shifted $V_{1/2}$ in a similar direction as the shift at V_{max}, although the shift was small and not significant, except for $Na_v1.8$. The shifts of $V_{1/2}$ by PAS were smaller than those by APAS. PAS significantly shifted $V_{1/2}$ in a depolarizing direction for $Na_v1.2$ and $Na_v1.6$ at $V_{1/2}$, but it had no or slight effects on all α subunits at V_{max}, and on $Na_v1.7$ and $Na_v1.8$ at $V_{1/2}$ (fig. 6 and tables 2 and 3).

Effects of APAS and PAS on Inactivation of Sodium Currents

We also investigated the effects of APAS and PAS on steady-state inactivation. Currents were elicited by a 50-ms test pulse to −20 mV for $Na_v1.2$ and $Na_v1.6$, −10 mV for $Na_v1.7$, and +10 mV for $Na_v1.8$ after 200 ms (500 ms for only $Na_v1.8$) prepulses ranging from −140 mV to 0 mV in 10-mV increments from V_{max} holding potential. Steady-state inactivation curves were fitted to the Boltzmann equation (see Electrophysiological Recordings under Materials and Methods). APAS and PAS significantly shifted the midpoint of steady-state inactivation ($V_{1/2}$) in the hyperpolarizing direction for all α subunits; APAS shifted by 8.0, 8.9, 6.7, and 8.9 mV and PAS shifted by 4.5, 8.0, 6.6, and 10.2 mV for $Na_v1.2$, $Na_v1.6$, $Na_v1.7$, and $Na_v1.8$, respectively (fig. 7 and tables 2 and 3). The effects of APAS and PAS in the hyperpolarizing range were consistent with the effects of these two neurosteroids on the peak I_{Na} at V_{max} and their effects on the I–V curves in the hyperpolarizing range at V_{max}.

Use-dependent Block of Sodium Currents by APAS and PAS

The use-dependent block of sodium currents by APAS and PAS was also investigated. Currents were elicited at 10 Hz by a 20-ms depolarizing pulse of −20 mV for $Na_v1.2$ and $Na_v1.6$ and −10 mV for $Na_v1.7$ from a $V_{1/2}$ holding potential in both the absence and presence of 100 μmol/l APAS and PAS. Peak currents were measured and normalized to the first pulse and plotted against the pulse number (fig. 8, A–D). Data were fitted by the monoexponential equation (see Electrophysiological Recordings under Materials and Methods). APAS significantly reduced the plateau I_{Na} amplitude of $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$ from 0.80 ± 0.03 to 0.57 ± 0.03, 0.89 ± 0.01 to 0.49 ± 0.07, and 0.89 ± 0.02 to 0.62 ± 0.06, respectively (fig. 8E). PAS also reduced the plateau I_{Na} amplitudes of $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$ from

Table 1. Fitted Parameters for Effects of APAS and PAS

	APAS			PAS		
	IC_{50}	EC_{50}	Hill Slope	IC_{50}	EC_{50}	Hill Slope
$Na_v1.2$	12.2±3.5		0.58±0.07	78.4±9.8		0.86±0.03
$Na_v1.6$	40.6±1.9		0.77±0.03	53.8±3.2		1.12±0.03
$Na_v1.7$	130.7±14.7		0.67±0.06	117.8±19.0		0.74±0.04
$Na_v1.8$		61.3±8.5	1.72±0.10		32.7±3.4	2.45±0.47

IC_{50} values, EC_{50} values, and Hill slopes calculated from nonlinear regression analyses of the dose–response curves shown in figure 4. Data are given as mean ± SEM; n = 6 ($Na_v1.2$), 7 ($Na_v1.6$), 5 ($Na_v1.7$), and 5 ($Na_v1.8$).

APAS = allopregnanolone sulfate; EC_{50} = half-maximal effective concentration; IC_{50} = half-maximal inhibitory concentration; Na_v = voltage-gated sodium channel; PAS = pregnanolone sulfate.

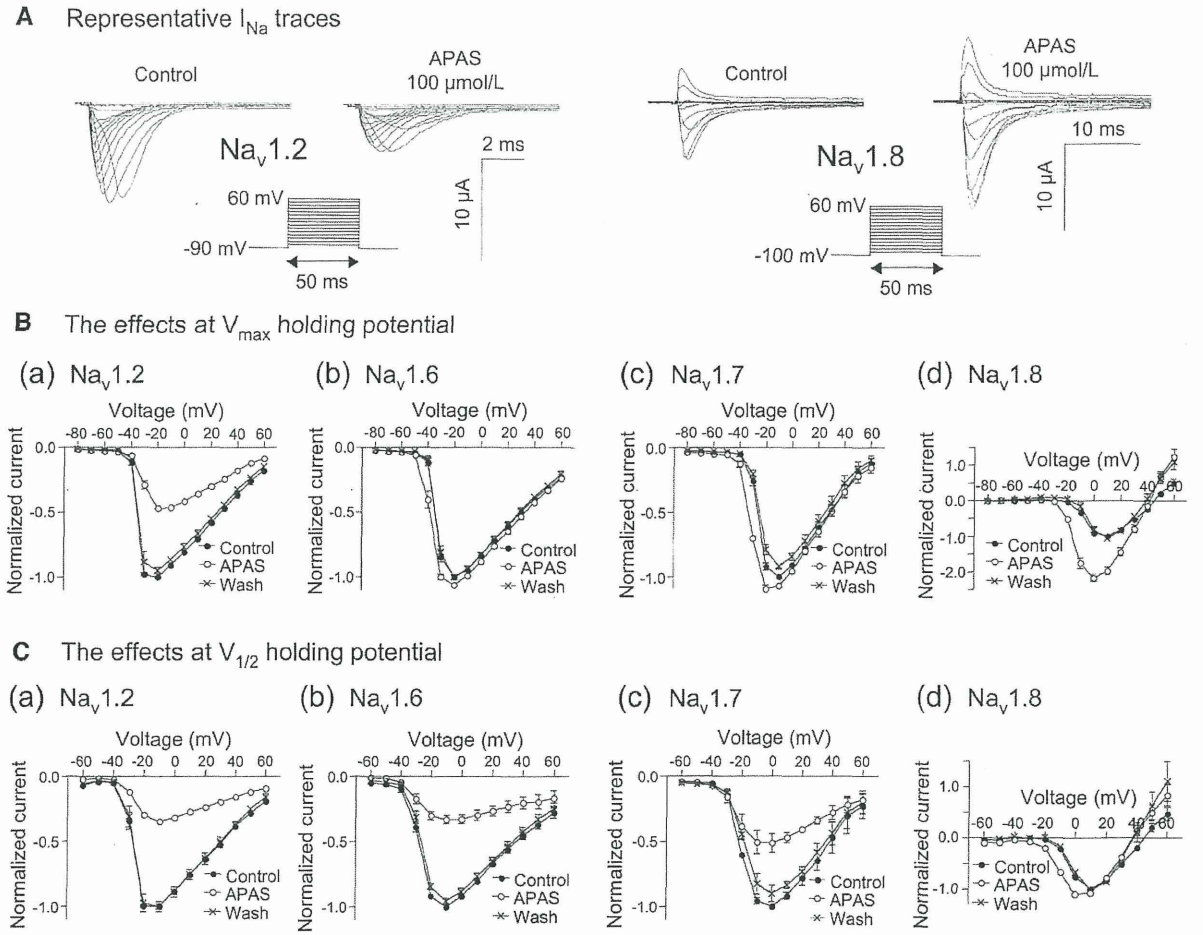

Fig. 5. Effects of allopregnanolone sulfate (APAS) on I–V curves of sodium currents in oocytes expressing $Na_v1.2$ (*a*) (n = 5), $Na_v1.6$ (*b*) (n = 7), $Na_v1.7$ (*c*) (n = 5), or $Na_v1.8$ (*d*) (n = 6) α subunits with β_1 subunits. Currents were elicited using 50-ms depolarizing steps between –80 and 60 mV in 10-mV increments from a V_{max} holding potential and elicited using 50-ms depolarizing steps between –60 and 60 mV in 10-mV increments from a $V_{1/2}$ holding potential. (*A*) Representative I_{Na} traces from oocytes expressing $Na_v1.2$ (*left*) and $Na_v1.8$ (*right*) with the β_1 subunit in both the absence and presence of 100 µmol/l of APAS at V_{max} holding potential are shown. The effects of APAS on normalized I–V curves elicited from V_{max} (*B*) and $V_{1/2}$ holding potentials (*C*) are shown (*closed circles*, control; *open circles*, neurosteroids; *cross*, washout). Peak currents were normalized to the maximal currents observed from –20 to +10 mV. Data are presented as means ± SEM. Na_v = voltage-gated sodium channel; V_{max} holding potential = holding potential causing maximal current; $V_{1/2}$ holding potential = holding potential causing half-maximal current; Wash = washout.

0.81±0.2 to 0.70±0.03, 0.94±0.01 to 0.73±0.02, and 0.91±0.02 to 0.75±0.01, respectively, and the reductions were significant except for $Na_v1.2$ (fig. 8F). These results demonstrated a use-dependent block of APAS and PAS on sodium channels, and the block by APAS was more potent than that by PAS.

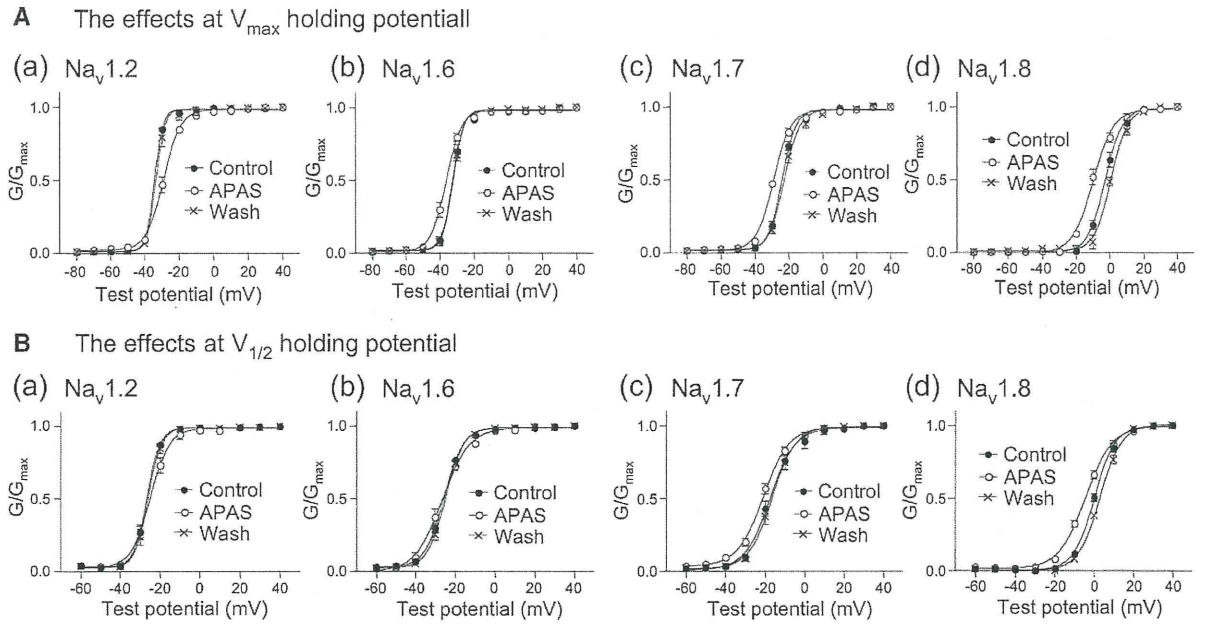

Fig. 6. Effects of allopregnanolone sulfate (APAS) on channel activation in oocytes expressing $Na_v1.2$ (*a*) (n = 5), $Na_v1.6$ (*b*) (n = 7), $Na_v1.7$ (*c*) (n = 5), or $Na_v1.8$ (*d*) (n = 6) α subunits with β_1 subunits from V_{max} (*A*) or $V_{1/2}$ holding potentials (*B*). *Closed circles*, *open circles*, and *cross* represent control, the effect of neurosteroids, and washout, respectively. Data are expressed as means ± SEM. Activation curves were fitted to the Boltzmann equation; $V_{1/2}$ is shown in table 2. Na_v = voltage-gated sodium channel; V_{max} holding potential = holding potential causing maximal current; $V_{1/2}$ holding potential = holding potential causing half-maximal current; Wash = washout.

Table 2. Effects of APAS on Activation and Inactivation

	$V_{1/2}$ (mV)					
	Holding V_{max}			Holding $V_{1/2}$		
	Control	APAS	Shift	Control	APAS	Shift
Activation						
$Na_v1.2$	−34.2 ± 0.5	−29.1 ± 1.0**	+5.1	−26.4 ± 0.8	−24.8 ± 1.1	+1.6
$Na_v1.6$	−32.5 ± 0.6	−36.3 ± 0.9***	−3.8	−25.6 ± 0.6	−26.7 ± 1.3	−1.1
$Na_v1.7$	−23.9 ± 0.6	−29.0 ± 0.3***	−5.1	−17.2 ± 1.7	−20.9 ± 0.9	−3.7
$Na_v1.8$	−2.7 ± 1.1	−9.8 ± 1.2***	−7.1	0.3 ± 0.6	−4.2 ± 0.8**	−4.5
Inactivation						
$Na_v1.2$	−50.1 ± 1.0	−58.1 ± 1.1***	−8.0			
$Na_v1.6$	−57.8 ± 0.5	−66.7 ± 0.7***	−8.9			
$Na_v1.7$	−72.3 ± 1.6	−79.0 ± 1.8***	−6.7			
$Na_v1.8$	−37.0 ± 2.2	−45.9 ± 1.7***	−8.9			

$V_{1/2}$ is calculated from nonlinear regression analyses of activation and inactivation curves shown in figures 6 and 7. Data are given as mean ± SEM; n = 5 ($Na_v1.2$), 7 ($Na_v1.6$), 5 ($Na_v1.7$), and 6 ($Na_v1.8$).

**P < 0.01; ***P < 0.001 compared with control, based on paired t test (two-tailed).

APAS = allopregnanolone sulfate; Holding V_{max} = holding potential causing maximal current; Holding $V_{1/2}$ = holding potential causing half-maximal current; Na_v = voltage-gated sodium channel; $V_{1/2}$ = the potential at which activation is half maximal for activation curve, and the voltage of half-maximal inactivation for inactivation curve.

Discussion

In the current study, we demonstrated that APAS and PAS differentially affected I_{Na} induced by four α subunits at both V_{max} and $V_{1/2}$ holding potentials. Moreover, we found that both neurosteroids suppress $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$ at $V_{1/2}$ in a concentration-dependent manner. IC_{50} values indicated that the effect of APAS on $Na_v1.2$ was most potent among the two compounds and three α subunits. To the best of our knowledge, this is the first direct evidence of the various effects of these two neurosteroids on neuronal sodium channel α subunits. It is thought that APAS is synthesized from allopregnanolone by 3α-hydroxysteroid

Table 3. Effects of PAS on Activation and Inactivation

	$V_{1/2}$ (mV)					
	Holding V_{max}			Holding $V_{1/2}$		
	Control	PAS	Shift	Control	PAS	Shift
Activation						
$Na_v1.2$	−33.4 ± 0.6	−30.5 ± 1.5	+2.9	−26.1 ± 0.9	−23.7 ± 1.1**	+2.4
$Na_v1.6$	−32.1 ± 0.5	−32.4 ± 0.8	−0.3	−24.8 ± 0.8	−20.7 ± 1.4**	+4.1
$Na_v1.7$	−23.2 ± 0.5	−23.9 ± 0.6	−0.7	−18.7 ± 1.0	−18.0 ± 0.9	+0.7
$Na_v1.8$	−1.4 ± 2.1	−2.3 ± 1.7	−0.9	−0.2 ± 0.8	−1.1 ± 0.9	−0.9
Inactivation						
$Na_v1.2$	−49.9 ± 0.8	−54.4 ± 1.5**	−4.5			
$Na_v1.6$	−57.5 ± 0.5	−65.5 ± 0.5***	−8.0			
$Na_v1.7$	−72.3 ± 1.0	−78.9 ± 1.0***	−6.6			
$Na_v1.8$	−36.0 ± 1.3	−46.2 ± 1.4**	−10.2			

$V_{1/2}$ is calculated from nonlinear regression analyses of activation and inactivation curves (not shown). Data are given as mean ± SEM; n = 6 ($Na_v1.2$), 7 ($Na_v1.6$), 5 ($Na_v1.7$), and 6 ($Na_v1.8$).

** $P < 0.01$; *** $P < 0.001$ compared with control, based on paired *t* test (two-tailed).

Holding V_{max} = holding potential causing maximal current; Holding $V_{1/2}$ = holding potential causing half-maximal current; Na_v = voltage-gated sodium channel; PAS = pregnanolone sulfate; $V_{1/2}$ = the potential at which activation is half maximal for activation curve, and the voltage of half-maximal inactivation for inactivation curve.

sulfotransferase *in vivo*, because 3α-hydroxysteroid sulfotransferase has been isolated *in vivo*.[26] Therefore, allopregnanolone likely exerts a portion of its effects through APAS, which is its metabolite.

It was reported that the level of endogenous allopregnanolone changes in many physiological and pathological situations within a serum concentration range of 1 to 10 nmol/l.[27,28] However, it is not clear whether allopregnanolone has an analgesic effect in physiological concentrations. A recent study demonstrated that 1 and 10 μmol/l of allopregnanolone reduced mechanical allodynia and thermal heat hyperalgesia in normal and neuropathic pain models in rats after 10-μl intrathecal injection.[29] Another investigator reported that intrathecal administration of 10 μmol/l of allopregnanolone showed antihyperalgesic effects in hyperalgesic rats after spinal nerve ligation.[30] From these previous studies, concentrations approximately 1 μmol/l allopregnanolone at receptive fields are estimated to have an analgesic effect. In the current study, APAS tended to, *albeit* not significantly, suppress the I_{Na} of $Na_v1.2$ at 0.3 μmol/l by 8% and significantly ($P < 0.01$) inhibited it at 1 μmol/l by 19 ± 2%. The IC_{50} value of $Na_v1.2$ inhibition by APAS was 12 μmol/l. It was reported that relatively small degrees of sodium channel inhibition could have profound effects on the neuronal firing rate because a 10% inhibition of sodium current reduces the number of action potentials to 10 from a control response of 21 in 750 ms.[24] Therefore, APAS may reduce neuronal firing for $Na_v1.2$ at a concentration exhibiting the antinociceptive effects of allopregnanolone in animal models, whereas the effects of APAS and PAS on another three α and four α subunits, respectively, may not be pharmacologically relevant because these effects were observed at concentrations over 10 μmol/l. In addition, the effects of highly hydrophobic compounds—such as neurosteroids—we used tend to be attenuated in the voltage-clamp techniques with *Xenopus* oocytes, compared with the whole-cell voltage-clamp methods using mammalian cells. Indeed, it was reported that the enhancing effect by allopregnanolone on $GABA_A$ receptor combination ($\alpha_1\beta_2\gamma_{2L}$) was more potent in the human embryonic kidney 293 cells system (EC_{50}; 41 ± 2 nmol/l)[31] than that in the *Xenopus* oocyte system (EC_{50}; 177 ± 2 nmol/l).[32] This may be a limitation of experiments using the *Xenopus* oocyte expression system; this limitation indicates that APAS might inhibit function of $Na_v1.2$ more potently in a mammalian cell system than in the oocyte system, however, it also could potentiate $Na_v1.8$ function more potently in a mammalian cell. Therefore, further investigation is needed to consider the roles of these α subunits in humans.

Analysis of gating revealed common characteristics but also some differences in the effects of APAS and PAS on different α subunits. A common effect on all α subunits was enhancement of inactivation. Because of this enhancement effect, the inhibitions by two compounds at $V_{1/2}$ holding potentials could be interpreted as stronger effects because they shift inactivation curve to the hyperpolarizing direction, which makes the channel into further inactivated state. In contrast, APAS enhanced peak I_{Na} at V_{max}, shifted activation in the hyperpolarizing direction, and increased sodium currents in the hyperpolarizing range of the inactivation curves for $Na_v1.6$, $Na_v1.7$, and $Na_v1.8$. These changes indicate that APAS shifts channel gating equilibrium toward the open channel state and activates sodium channels. This action might attenuate the effects on the inactivated state and, especially, lead to enhancement of I_{Na} even in the inactivated state ($V_{1/2}$ holding potential) for $Na_v1.8$ in spite of the great enhancement of inactivation. However, for $Na_v1.2$, APAS profoundly suppressed peak I_{Na} at V_{max}, shifted activation in the depolarizing direction at V_{max}, and greatly decreased

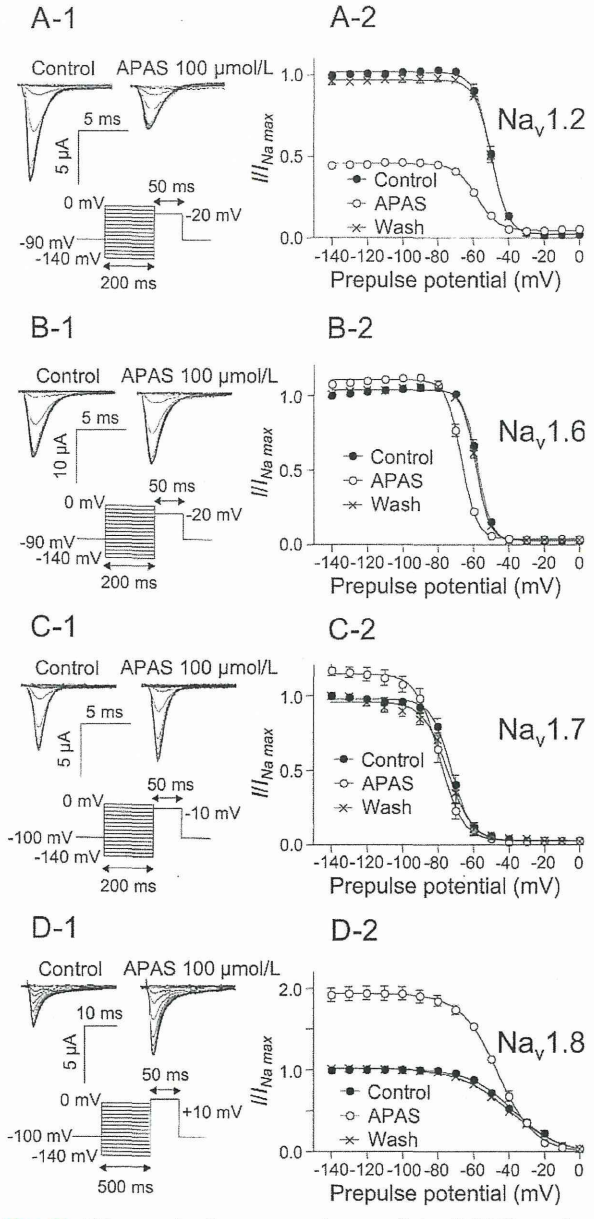

Fig. 7. Effects of allopregnanolone sulfate (APAS) on inactivation curves in oocytes expressing $Na_v1.2$ (*A*) (n = 6), $Na_v1.6$ (*B*) (n = 7), $Na_v1.7$ (*C*) (n = 5), or $Na_v1.8$ (*D*) (n = 6) α subunits with β_1 subunits. Currents were elicited by a 50-ms test pulse to −20 mV for $Na_v1.2$ and $Na_v1.6$, −10 mV for $Na_v1.7$, and +10 mV for $Na_v1.8$ after 200 ms (500 ms for only $Na_v1.8$) prepulses ranging from −140 mV to 0 mV in 10-mV increments from a V_{max} holding potential. Representative I_{Na} traces in both the absence and presence of APAS are shown in *A-1*, *B-1*, *C-1*, and *D-1*. Effects of APAS on inactivation curves (*closed circles*, control; open circles, neurosteroids; cross, washout) are shown in *A-2*, *B-2*, *C-2*, and *D-2*. Steady-state inactivation curves were fitted to the Boltzmann equation, and the $V_{1/2}$ values are shown in table 2. Data are expressed as means ± SEM. Na_v = voltage-gated sodium channel; Wash = washout.

sodium currents in the hyperpolarizing range of the inactivation curve, indicating that resting channel block is an important mechanism of APAS inhibition for only $Na_v1.2$. Both compounds demonstrated use-dependency for inhibition of $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$, suggesting the ability to slow the recovery time from inactivation.[33] Many investigators have shown that sodium channel blockers, including local anesthetics, tricyclic antidepressants, and volatile anesthetics, enhance steady-state inactivation with no effect on activation and exhibit use-dependent block.[34–36] We demonstrated that APAS enhances inactivation and shows use-dependent block similar to other sodium channel blockers, yet it also has diverse effects on activation according to differences in α subunits. These actions suggest that APAS may have different binding sites or allosteric conformational mechanisms to change sodium channel function, although further investigation with site-directed mutagenesis is needed to rule out nonspecific membrane effects. PAS may have common binding sites with APAS, because it shows similar effects, although these changes were small.

The α subunit consists of four homologous domains (I to IV) containing six transmembrane segments (S1 to S6), and one reentrant P-region connecting S5 to S6 (SS1/SS2). Tetrodotoxin-sensitive α subunits, $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$, are phylogenetically related and show 70 to 80% amino acid sequence identity. In contrast, tetrodotoxin-resistant α subunits, $Na_v1.8$, are phylogenetically distant and show only 55 to 56% sequence identity to the other three α subunits. In addition, the lengths of amino acid sequences of four α subunits differed within the range of 1957 to 2005 residues. Therefore, these differences would result in the diversity in neurosteroid action, especially in the effects on channel activation. Indeed, the longest extracellular regions in the α subunit (IS5 to SS1) are 93, 77, 73, and 66 amino acid residues in $Na_v1.2$, $Na_v1.6$, $Na_v1.7$, and $Na_v1.8$, respectively. The diversity in sequence and differences in the effects on activation according to α subunit may be important for clarifying binding sites and the mechanism of $Na_v1.2$ inhibition by APAS in further investigations.

γ-Aminobutyric acid type A receptors have been considered to be important for the analgesic effects of allopregnanolone because it has high potency as a positive $GABA_A$ modulator compared with other neurosteroids. Pregnanolone also affects $GABA_A$ receptors in a manner similar to that of allopregnanolone; nevertheless, its analgesic effect is weak. In fact, pregnanolone was shown to reduce mechanical allodynia without reduction of thermal heat hyperalgesia in a neuropathic pain model in contrast to attenuation of both by allopregnanolone.[28] The investigators suggested that the partial analgesic effects of pregnanolone are caused by suppression of glycine receptors by demonstrating that pregnanolone had a significant analgesic effect only in animals displaying a strychnine-induced allodynia in two types of allodynia models induced by bicuculline and strychnine.[28] Moreover, a recent report demonstrated that

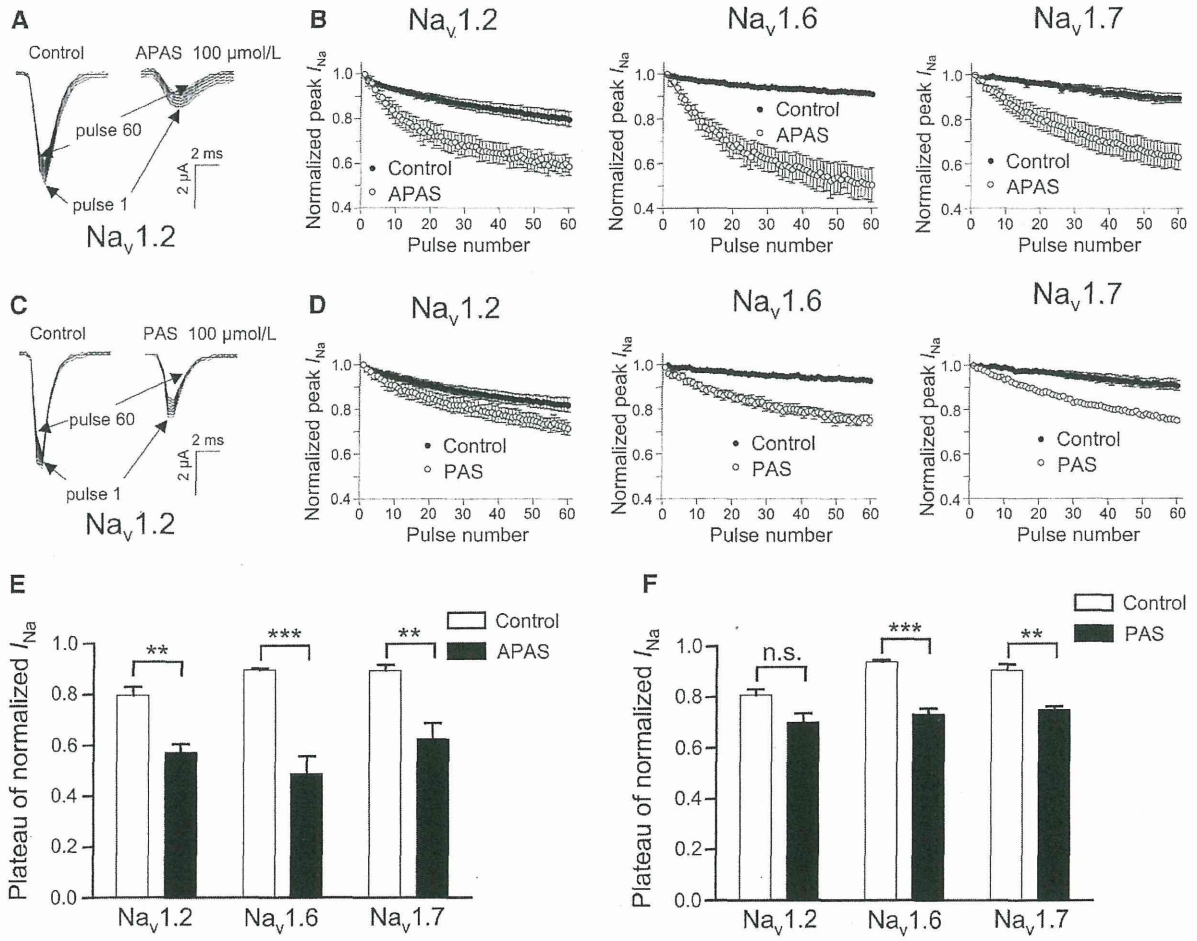

Fig. 8. Use-dependent blockage of sodium channels on Na$_v$1.2 (n = 5), Na$_v$1.6 (n = 6), and Na$_v$1.7 (n = 5) α subunits with β_1 subunits by allopregnanolone sulfate (APAS) and pregnanolone sulfate (PAS). Currents were elicited at 10 Hz by a 20-ms depolarizing pulse of –20 mV for Na$_v$1.2 and Na$_v$1.6 and –10 mV for Na$_v$1.7 from a $V_{1/2}$ holding potential in both the absence and presence of 100 μmol/l of the two compounds; representative I_{Na} traces in both the absence and presence of the two compounds (*A* and *C*). Peak currents were measured and normalized to the first pulse and plotted against the pulse number (*B*, the effects of APAS; *D*, the effects of PAS). *Closed circles* and *open circles* represent control and the effect of neurosteroids, respectively. Data were fitted to the monoexponential equation, and values for fractional blockage of the plateau of normalized I_{Na} are shown in *E* and *F*. Data are expressed as means ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the control, based on paired *t* test (two-tailed). Na$_v$ = voltage-gated sodium channel.

allopregnanolone shows analgesic effects in rats through suppression of T-type Ca^{2+} currents and potentiation of $GABA_A$ currents.[16] These previous reports indicate several mechanisms underlying the analgesic effect of allopregnanolone likely exist, as well as potentiation of $GABA_A$ receptors.

Sodium channel α subunits expressed in the dorsal root ganglion (Na$_v$1.7, Na$_v$1.8, and Na$_v$1.9) are thought to be involved in the pathogenesis of inflammatory and neuropathic pain. A recent study reported that Na$_v$1.2 also plays an important role in pain signaling. It was reported that Na$_v$1.2 and Na$_v$1.3 predominantly compose functional sodium channel currents within lamina I/II (dorsal horn) neurons, which mediate acute and chronic nociceptive signals from peripheral nociceptors to pain-processing regions in the brain.[37] Another recent report showed that mutations in Na$_v$1.2 are associated with seizures and pain characterized by headaches and back pain.[38] A disubstituted succinamide, a potent sodium channel blocker, was reported to attenuate nociceptive behavior in a rat model of tonic pain and was demonstrated to potently block Na$_v$1.2, as well as Na$_v$1.7 and Na$_v$1.8, with a potency two orders of magnitude higher than anticonvulsant and antiarrhythmic sodium channel blockers currently used to treat neuropathic pain.[39] Other investigators demonstrated that four sodium channel blockers, including lidocaine, mexiletine, benzocaine, and ambroxol, which are used clinically to treat pain, suppressed recombinant Na$_v$1.2 currents as well as tetrodotoxin-resistant Na^+ channel currents in rat sensory neurons, which comprised mostly Na$_v$1.8 currents. The authors suggested that these sodium channel blockers would induce analgesia according

to the amount of sodium channel blocking, including $Na_v1.2$ and $Na_v1.8$.[40] These recent reports support that suppression of $Na_v1.2$ function by APAS might be a mechanism underlying the analgesic effects of allopregnanolone.

In conclusion, APAS and PAS have diverse effects on $Na_v1.2$, $Na_v1.6$, $Na_v1.7$, and $Na_v1.8$ α subunits expressed in *Xenopus* oocytes, with differences in the effects on sodium channel gating. In particular, only APAS inhibited sodium currents of $Na_v1.2$ at pharmacologically relevant concentrations. These results raise the possibility that suppression of $Na_v1.2$ by APAS may be important for pain relief by allopregnanolone and provide a better understanding of the mechanisms underlying the analgesic effects of allopregnanolone. However, further studies are needed to clarify the relevance of sodium channel inhibition by APAS.

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Competing Interests

The authors declare no competing interests.

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Cancer Cachexia Pathophysiology and Translational Aspect of Herbal Medicine

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About half of all cancer patients show a syndrome of cachexia, characterized by anorexia and loss of adipose tissue and skeletal muscle mass. Numerous cytokines have been postulated to play a role in the etiology of cancer cachexia. Cytokines can elicit effects that mimic leptin signaling and suppress orexigenic ghrelin and neuropeptide Y signaling, inducing sustained anorexia and cachexia not accompanied by the usual compensatory response. Furthermore, cytokines have been implicated in the induction of cancer-related muscle wasting. In particular, tumor necrosis factor-alpha, interleukin-1, interleukin-6 and interferon-gamma have been implicated in the induction of cancer-related muscle wasting. Cytokine-induced skeletal muscle wasting is probably a multifactorial process, which involves a depression in protein synthesis, an increase in protein degradation or a combination of both. Cancer patients suffer from the reduction in physical function, tolerance to anti-cancer therapy and survival, while many effective chemotherapeutic agents for cancer are burdened by toxicities that can reduce patient's quality of life or hinder their effective use. Herbal medicines have been widely used to help improve such conditions. Recent studies have shown that herbal medicines such as *rikkunshito* enhance ghrelin signaling and consequently improve nausea, appetite loss and cachexia associated with cancer or cancer chemotherapy, which worsens the quality of life and life expectancy of the patients. The multicomponent herbal medicines capable of targeting multiple sites could be useful for future drug discovery. Mechanistic studies and identification of active compounds could lead to new discoveries in biological and biomedical sciences.

Key words: appetite loss – muscle wasting – cytokine – ghrelin – palliative cancer treatment – herbal medicine

INTRODUCTION

Cancer patients suffer from weight loss and appetite loss, as well as from the reduction in physical function, tolerance to anti-cancer therapy and survival that are related to cachexia in advanced cancer (1). Cachexia is a debilitating state of involuntary weight loss complicating malignant, infectious and inflammatory diseases and contributing significantly to mortality (2). The word 'cachexia' is derived from the Greek words 'kakos' meaning 'bad' and 'hexis' meaning 'condition' (3). Anorexia, involuntary weight loss, tissue wasting, poor performance and ultimately death characterize cancer cachexia—a condition of advanced protein calorie malnutrition (2–7). Referred to as 'the cancer anorexia–cachexia syndrome', anorexia, or loss of compensatory increase in