Effects of General Anesthetics on Excitatory and Inhibitory Synaptic Transmission in Area CA1 of the Rat Hippocampus *In Vitro*

Masahiro Wakasugi

Department of Anesthesiology, Faculty of Medicine,

Toyama Medical and Pharmaceutical University

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Abstract

It is unclear whether general anesthetics induce enhancement of neural inhibition and/or attenuation of neural excitation. We studied the effects of pentobarbital ($5x10^{-4}$ mol/L), propofol ($5x10^{-4}$ mol/L), ketamine (10^{-3} mol/L), halothane (1.5 vol%) and isoflurane (2.0 vol%) on both excitatory and inhibitory synaptic transmission in rat hippocampal slices. Excitatory or inhibitory synaptic pathways were isolated using pharmacological antagonists. Extracellular microelectrodes were used to record electrically evoked CA1 neural population spikes (PSs). In the presence of a γ - aminobutyric acid type A ($GABA_A$) receptor antagonist (bicuculline), the inhibitory actions of pentobarbital and propofol on PSs were completely eliminated, while those of ketamine, halothane and isoflurane were only partially attenuated. In order to isolate the N-methyl-D-aspartate (NMDA) receptor mediated PS (NMDA PS), non-NMDA glutamate receptors and $GABA_A$ receptors were blocked by their antagonists in the absence of Mg^{2+}. Ketamine, halothane and isoflurane decreased the NMDA PS, and pentobarbital and propofol had no effect on it. The non-NMDA receptor mediated PS (non-NMDA PS) was examined using the antagonists for NMDA and $GABA_A$ receptors. Volatile but not iv anesthetics reduced the non-NMDA PS. These findings indicate (a) that pentobarbital and propofol produce inhibitory actions on PSs due to enhancement of the $GABA_A$ receptor - mediated response, (b) that ketamine reduces NMDA receptor- and enhances $GABA_A$ receptor-mediated responses, and (c) that halothane and isoflurane depress all of $GABA_A$ receptor-, NMDA receptor- and non-NMDA receptor-mediated synaptic transmission.

Implications: Volatile anesthetics depress both excitatory and inhibitory synaptic transmission of *in vitro* rat hippocampal pathways, whereas iv anesthetics produce more specific actions on inhibitory synaptic events. These results provide further support that general anesthetics produce drug-specific and selective effects on different pathways in the central nervous system.

key words

1) Anesthetics, Intravenous: pentobarbital, propofol, ketamine
2) Anesthetics, Volatile: halothane, isoflurane
3) Receptors: GABA, NMDA, non-NMDA
4) Synapse
5) Brain: hippocampus

Introduction

Although the mechanisms underlying general anesthesia are not well elucidated, it is possible that the anesthetic state can be achieved by enhancing neuronal inhibition, by decreasing neuronal excitation, or by a combination of both. Since Nicoll [1] initially suggested that general anesthetics enhance inhibitory synaptic transmission, γ -aminobutyric acid (GABA) mediated synaptic inhibition has been investigated as a target site for these drugs. Enhancement of the GABA receptor channel response may be a primary action of volatile anesthetics [2] whereas various iv anesthetics enhance GABA-mediated inhibition [3, 4].

A large number of studies have investigated the effects of general anesthetics on excitatory synaptic transmission. Richards [5] was the first to demonstrate that halothane depressed excitatory synaptic transmission in central nervous system preparations *in vitro*. It was later shown that halothane depress glutamate receptor-mediated excitatory postsynaptic currents using patch-clamp techniques [6]. MacIver et al. demonstrated that volatile anesthetics depressed glutamatergic synaptic transmission via reduction of presynaptic glutamate release in CA1 neurons of rat hippocampal slices[7].

These findings imply that not all general anesthetics affect excitatory and inhibitory synaptic transmission in the same manner. To our knowledge there have not been any reports compared the effects of various anesthetics on both excitatory and inhibitory synaptic transmission using identical preparation. In the present study, we pharmacologically isolated excitatory and inhibitory synaptic pathways in the area CA1

of rat hippocampus *in vitro*, and have then examined the effects of various intravenous and volatile anesthetics under these conditions.

Material and Method

Solution and Drugs: The composition of the artificial cerebrospinal fluid (ACSF) was (mmol/L): NaCl 124, KCl 5, NaH_2PO_4 1.25, $CaCl_2$ 2, $MgSO_4$ 2, $NaHCO_3$ 26, Glucose 10, made with distiled (18M Ω purity) water. ACSF was pre-cooled (8-10℃) and equilibrated with 95% oxygen / 5% carbon dioxide gas mixture before use (pH 7.35-7.45). The Mg^{2+}-free ACSF was identical to the ACSF except that $MgSO_4$ was omitted. Pentobarbital, ketamine and halothane were purchased from Dinabot (Osaka, Japan), Sigma (St. Louis, MO, USA), and Takeda (Osaka, Japan), respectively. Isoflurane and propofol were kindly donated from Dinabot and Zeneca (Cheshire, UK), respectively. CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) was obtained from Tocris Cookson (Bristol, UK). Other chemicals used were obtained from Sigma.

Preparation of Hippocampal Slices: The technique for the preparation of rat hippocampal slices was identical to the method described previously by Hirota & Roth [8]. After approval had been obtained from the Animal Research Committee of Toyama Medical and Pharmaceutical University, male Wistar rats (100-200g) were deeply anesthetized with sevoflurane and decapitated. The brain was rapidly removed, and the dissected hippocampus was sliced in cold ACSF (8-10℃) transversely to its long axis (400μm thick) with a microslicer (Dosaka EM, Osaka, Japan). Slices were placed onto a

nylon mesh at a liquid-gas interface in a recording chamber at 37℃. A humidified gas mixture (95%O_2/5%CO_2) was applied to the chamber at a rate of 1L/min and ACSF was continuously perfused at a rate of 90 ml/hour.

Recording and Stimulation: Square-wave paired-pulse stimuli (5-10 volt, 0.05 ms, 40 ms interval, 0.1 Hz), generated with a SEN-7203 stimulator (Nihon Kohden, Tokyo, Japan), were delivered to Schaffer collateral fiber via a nichrome bipolar electrode. The minimal stimulus intensity that elicited a maximal population spike amplitude ("maximal stimulus") was used. Extracellular recordings were made with the 2 mol/L NaCl filled glass microelectrodes (3-6 MΩ) placed in the area of CA1 cell bodies. Evoked responses were amplified (low -and high-cut filters set at 1 Hz and 10 kHz, respectively) with a MEZ-8301 amplifier (Nihon Kohden, Tokyo, Japan) and A/D conversions were made at a rate of 14,400 Hz using a MacADIOS (GW, MA, USA). Data were stored on a hard disk of a Macintosh computer for later analysis.

Drugs Administration: Pentobarbital, ketamine, bicuculline and AP5 were dissolved in ACSF at required concentrations. Stock solutions of CNQX (10^{-3} mol/L) was prepared in dimethyl sulfoxide (DMSO), and propofol was dissolved in 10% Intralipid (Pharmacia AB, Stockholm, Sweden) at a concentration of 10 mg/ml. These stock solutions were diluted in ACSF prior to perfusion into the chamber. The concentrations of DMSO and Intralipid utilized in the experiments did not affect the field potentials. Volatile anesthetics, halothane and isoflurane, were applied as vapors to the chamber with 95%O_2/5%CO_2 gas mixture, using a vaporizer, previously calibrated with an anesthetic gas analyzer (Capnomac; Datax, Finland). The concentrations of

volatile anesthetics, expressed as volume percent (vol %), refer to the dial settings on the vaporizer. All drugs were applied for 20 minutes prior to recording in order to obtain stable effects. The Mg^{2+}-free ACSF was used in experiments examining NMDA receptor-mediated responses since Mg^{2+} has been reported to block the NMDA channel at negative membrane potentials [10]. The iv anesthetic concentrations applied to *in vitro* preparations were calculated based on the method previously described by Richards [11]. The doses of pentobarbital, propofol and ketamine required to anesthetize experimental animals range from 20-30 mg/kg [11], 10-24 mg/kg [4] and 44-250 mg/kg [12]. Since the iv anesthetics can be considered diluted by the extracellular fluid (20-30% of the total body weight), these amounts of pentobarbital, propofol and ketamine should have maximum concentrations in the extracellular fluid in the ranges of $3\text{-}5\text{x}10^{-4}$ mol/L, $2\text{-}6\text{x}10^{-4}$ mol/L and $2\text{-}10\text{x}10^{-4}$ mol/L. On the basis of these calculations the concentration-response curves generated in preliminary experiments, and the calculated ED_{50} values of anesthetics were then tested in the current study: pentobarbital $5\text{x}10^{-4}$ mol/L, propofol $5\text{x}10^{-4}$ mol/L, ketamine 10^{-3} mol/L, halothane 1.5 vol% and isoflurane 2.0 vol%.

Evaluation of Inhibitory and Excitatory Synaptic Transmission: The extent to which inhibitory synaptic transmission contributes to depression of population spikes (PSs) were studied in the presence of the $GABA_A$ receptor antagonist, bicuculline methiodide (BMI, $5\text{x}10^{-5}$ mol/L). Two types of ionotropic glutamate receptors were pharmacologically isolated using specific receptor antagonists [13]. In order to assess the NMDA receptor-mediated PS (NMDA PS), the $GABA_A$ receptor antagonist (BMI,

$5x10^{-5}$ mol/L) and the non-NMDA receptor antagonist (CNQX, 10^{-6} mol/L) were applied under Mg^{2+}-free conditions. The $GABA_A$ receptor antagonist and the NMDA receptor antagonist AP-5 (DL-2-amino-5-phosphonovaleric acid, $5x10^{-5}$ mol/L) were used to evaluate the non-NMDA receptor-mediated PS (non-NMDA PS).

Data Analysis: PS amplitudes were measured for evaluation in a similar manner as previously described [8]. Measurements were made from onset to the peak of the waveform. For data analysis, five evoked waves were collected and averaged. The effects of the majority of anesthetics were determined on the first evoked PSs (PS1s) in the current experiments, however, the second evoked PSs (PS2s) were used for pentobarbital and propofol, since our previous studies have revealed that iv anesthetics produce greater effects on PS2 than PS1 in identical preparations [9]. Statistical significance of the data was determined using ANOVA followed by Bonnferroni test. A P value less than 0.05 was considered significant. Data are expressed as mean±S.D.

Results

Effects of General Anesthetics on Field Potentials: In control conditions (no anesthetic), the amplitude of PS2 was larger than that of PS1. Pentobarbital and propofol decreased PS2 with a minimal change in PS1. Ketamine decreased both PS1 and PS2 to the same extent, whereas the volatile anesthetics, isoflurane and halothane, had greater effects on PS1 compared to PS2 (Table; Fig. 1, ACSF). The effects were completely recovered upon washout.

Effects of General Anesthetics on GABAergic Inhibition: The administration of BMI induced multiple spikes and enhanced the PS1 and PS2 amplitudes to 107.6±6.4% and 106.8±6.1% of control (n=6), respectively (Fig. 1, +BMI). The inhibitory effects of pentobarbital and propofol on PSs were completely eliminated with BMI, while the effects of ketamine, halothane and isoflurane were only partially suppressed (Fig. 2). The results indicate that the inhibitory effects of pentobarbital and propofol are mainly due to the $GABA_A$-mediated mechanisms. Since other factors may be involved in the actions of ketamine and volatile anesthetics, the following experiments on excitatory synaptic transmission were performed.

Effects of General Anesthetics on NMDA Receptors: The administration of CNQX in the absence of Mg^{2+} reduced the amplitudes of PS1 and PS2 to 77.0±7.5% and 89.1±6.7% of control, respectively (n=5). BMI added to the Mg^{2+}-free ACSF containing CNQX induced multiple spikes without significant changes in amplitudes of PS1 and PS2. Under these conditions, PSs were elicited via NMDA receptor-mediated synaptic transmission. Pentobarbital and propofol had no significant effect on NMDA PSs, while ketamine, halothane and isoflurane significantly decreased NMDA PSs (Fig. 3A).

Effects of General Anesthetics on Non-NMDA Receptor: AP-5 alone had no consistent effect on the shape of PS, and the application of AP-5 and BMI in ACSF enhanced the PS1 and PS2 amplitudes to 121.7±8.7% and 120.1±6.6% of control, respectively (n=5). The non-NMDA PSs were not affected by pentobarbital, propofol and ketamine, but were significantly decreased with volatile anesthetics (Fig. 3B).

Discussion

The hippocampus is a highly laminated limbic structure with well-defined afferents, efferents and neurotransmitters, and may be one of the major target sites for general anesthetics [14]. Thus the hippocampal slice preparation is an ideal model system for the study of anesthetic action on synaptic transmission in the central nervous system. In the present study we evoked PSs in area CA1 of rat hippocampus via stimulation of the Schaffer collateral fibers. This pathway is monosynaptic and is inhibited via recurrent inhibitory interneurons. Since PS reflects the number and synchrony of pyramidal cells that generate action potentials, the gradual decline of neural excitability (run down) is expected to be minimal.

The concentrations of iv anesthetics tested in the current study were higher than the plasma concentrations in clinical settings. Since the doses of iv anesthetics to anesthetize experimental animals are 10 to 100 times higher than those for humans [4, 11, 12], the different sensitivities to iv anesthetics between species (see Method) could be involved. It might be attributed to the limitations of the *in vitro* preparations: missing of the blood-born factors from ACSF and/or lack of certain inputs and outputs normally existing in the intact brain. The same degree of iv anesthetic concentrations [11, 12] have been actually employed for *in vitro* electrophysiological studies in brain slice preparations.

GABA is the major inhibitory neurotransmitter in hippocampus. The $GABA_A$ receptor (the BMI sensitive receptor) is a ligand-gated ion channel consisting of a

chloride channel complex. When the postsynaptic $GABA_A$ receptor is activated, chloride ions move into the postsynaptic cell causing an increased membrane conductance that inhibits postsynaptic action potential discharge and decreases excitatory synaptic responses. Glutamate is the major excitatory neurotransmitter in the hippocampus. Glutamate receptors are divided into two functional subtypes (NMDA and non-NMDA) by their specific agonists. Glutamate released into the synaptic cleft activates both postsynaptic NMDA and non-NMDA receptors and induces an excitatory postsynaptic current (EPSC). The fast component of EPSC is due to the non-NMDA receptor and the slower component is attributed to the NMDA receptor.

In the current study we have demonstrated for the first time in the same preparation that there are different actions of general anesthetics on inhibitory and excitatory synaptic events in the central nervous system. Pentobarbital and propofol augment $GABA_A$ receptor-mediated inhibitory synaptic transmission but not NMDA and non-NMDA receptor-mediated excitatory synaptic transmission. The results are in agreement with previous reports [3,4]. It has previously been shown that pentobarbital can block NMDA receptor-mediated currents in isolated single hippocampal neurons [15]. Although the excitatory synaptic transmission might be altered, the current experiments provide evidence that pentobarbital has a primary action on inhibitory rather than excitatory synaptic transmission.

We found that ketamine depresses NMDA PS but not non-NMDA PS, indicating that this anesthetic inhibits excitatory synaptic transmission via NMDA receptors as previously reported [16]. The effects of ketamine on GABA receptors are controversial.

Tang & Schroeder [17] and Brockmeyer & Kendig [18] reported that ketamine does not attenuate GABAergic synaptic transmission in the spinal cord. We observed, however, that BMI partially depressed the actions of ketamine, suggesting that the action of the anesthetic could be, in part, a result of enhancement of GABAergic inhibitory synaptic transmission. Our results are consistent with other studies in the cervical ganglion [19] and hippocampus [20]. Since ketamine has different regional actions on the NMDA receptor [21], it is possible that ketamine could produce different actions on GABAergic transmission in the hippocampus compared to the spinal cord.

The present results demonstrated that volatile anesthetics exerted enhancement of the inhibitory synaptic transmission and reduction of the excitatory synaptic transmission. It has been reported that post synaptic $GABA_A$ receptors are considered as a main target of general anesthetics [2] and recently Mimic et al. [22] identified that there are the specific sites on $GABA_A$ receptors modulated by volatile anesthetics. Recent studies, however, propose that volatile anesthetics attenuate the glutamate receptor-mediated synaptic transmission in the central nervous system [5-7, 13].

Our experiments were based on the fact that the Schaffer collateral input to CA1 pyramidal neurons of the hippocampus is generated via glutamate-mediated monosynaptic excitatory synaptic transmission in combination with $GABA_A$ergic recurrent inhibition. It has also been reported that volatile anesthetics augment the $GABA_B$ receptor-mediated inhibition in hippocampus [8] and that volatile anesthetics can depress postsynaptic sodium channels [23] and calcium channels [24]. Thus, there is the possibility that a number of receptor-mediated pathways and/or post synaptic

events may be involved in the actions of volatile anesthetics.

In conclusion the present study has shown that general anesthetics can produce different effects on GABAergic inhibitory and glutamatergic excitatory synaptic transmission in the hippocampal pyramidal cell. Volatile anesthetics modulate both excitatory and inhibitory synaptic activities, whereas iv anesthetics produce more specific actions on inhibitory synaptic events. These result support the hypothesis of drug and site-specific mechanisms of general anesthesia [14, 25].

We thank Zeneca, Cheshire, UK, for the gift of propofol; and Dinabot, Osaka, Japan, for the gift of isoflurane and a isoflurane vaporizer.

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Table. The effects of general anesthetics on population spikes in CA1 neurons induced by paired - pulse stimuli

Anesthetic (n)	Dose	PS 1	PS 2
Pentobarbital (4)	5×10^{-4} mol/L	107.2 ± 2.2	49.9 ± 3.9 *
Propofol (6)	5×10^{-4} mol/L	97.2 ± 5.4	56.7 ± 6.7 *
Ketamine (6)	10^{-3} mol/L	50.7 ± 4.9 *	53.9 ± 3.3 *
Halothane (5)	1.5 vol %	55.5 ± 6.5 *	71.3 ± 9.8 *
Isoflurane (5)	2.0 vol %	56.8 ± 6.4 *	71.2 ± 5.3 *

The population spikes were electrically elicited via Schaffer collateral fibers with paired-pulse stimuli (5-10 volt, 0.05 ms, 40ms interval, 0.1Hz). PS1: the first evoked population spike, PS2: the second evoked population spike. Results are expressed as percent of control (mean±SD). *P<0.05 compared with control

Figure Legends

Figure 1.

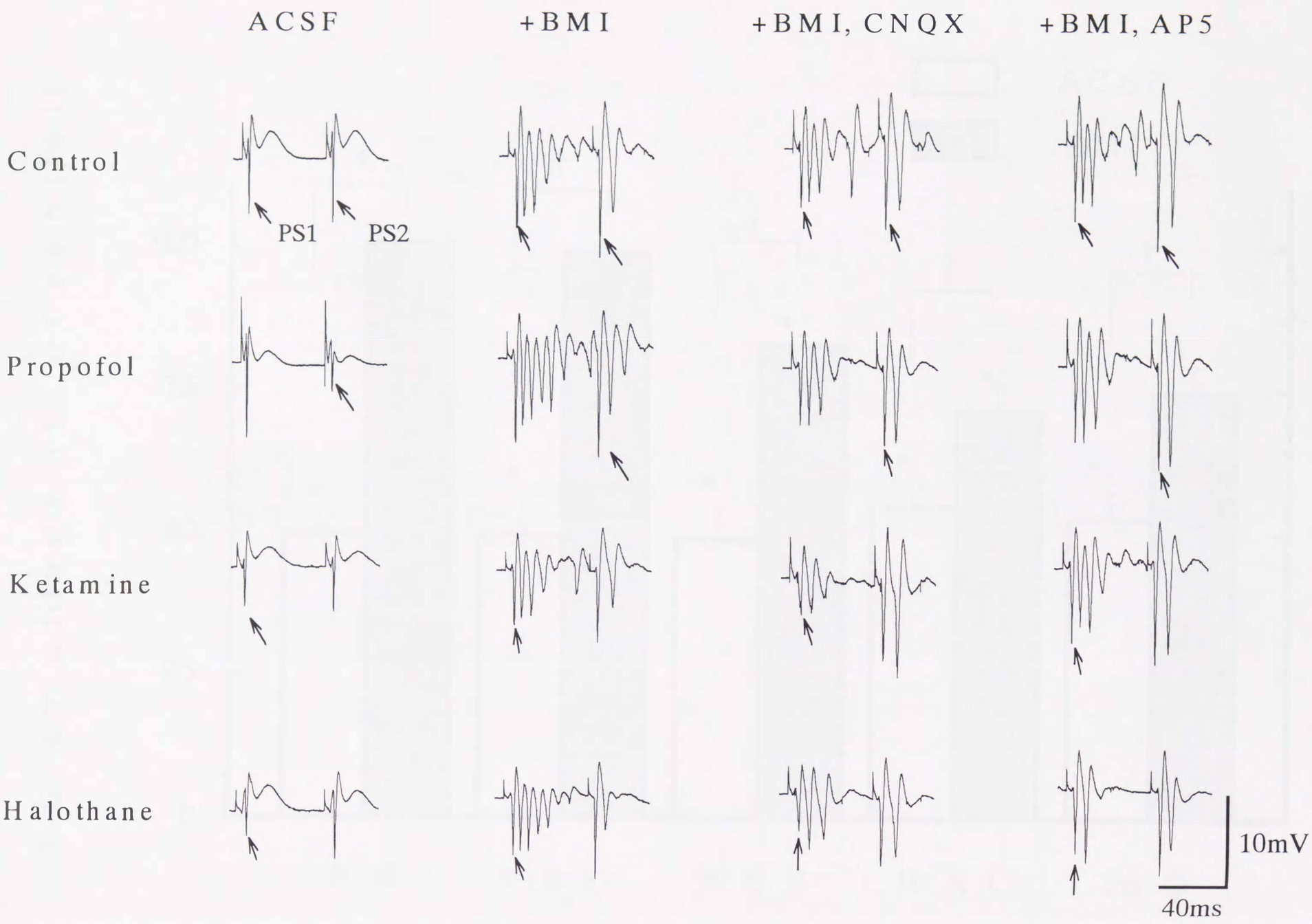
The effects of propofol ($5x10^{-4}$ mol/L), ketamine (10^{-3} mol/L) and halothane (1.5 vol%) on the evoked population spikes (PSs) in the absence and presence of specific receptor antagonists. PSs were elicited with a paired-pulse stimulus (5-10 volt, 0.05 ms, 40 ms interval) at 0.1 Hz. The initial PS amplitudes (indicated by arrows) were used for data analysis. ACSF: artificial cerebrospinal fluid, BMI: bicuculline methiodide ($5x10^{-5}$ mol/L), AP-5: DL-2-amino-5-phosphonovaleric acid (10^{-5} mol/L), CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione (10^{-6} mol/L)

Figure 2.

Effects of general anesthetics on PS amplitudes in the artificial cerebrospinal fluid (ACSF) and the presence of $GABA_A$ receptor antagonist, bicuculline methiodide (BMI, $5x10^{-5}$ mol/L). Each bar represents Mean±SD (% of control). PB: pentobarbital ($5x10^{-4}$ mol/L), n=4; PRO: propofol ($5x10^{-4}$ mol/L), n=6; KET: ketamine (10^{-3} mol/L), n=6; HAL: halothane (1.5 vol%), n=5; ISO: isoflurane (2.0 vol%), n=5. *$P<0.05$ compared with data in the absence of anesthetics; †$P<0.05$ compared with data in the absence of BMI.

Figure 3.

Effects of general anesthetics on the NMDA PS (A) and the non-NMDA PS (B). Each bar represents percent of control (Mean±SD). PB: pentobarbital ($5x10^{-4}$ mol/L), n=4; PRO: propofol ($5x10^{-4}$ mol/L), n=6; KET: ketamine (10^{-3} mol/L), n=6 ; HAL: halothane (1.5 vol%), n=5; ISO: isoflurane (2.0 vol%), n=5. *$P<0.05$ compared with the data in the absence of anesthetics.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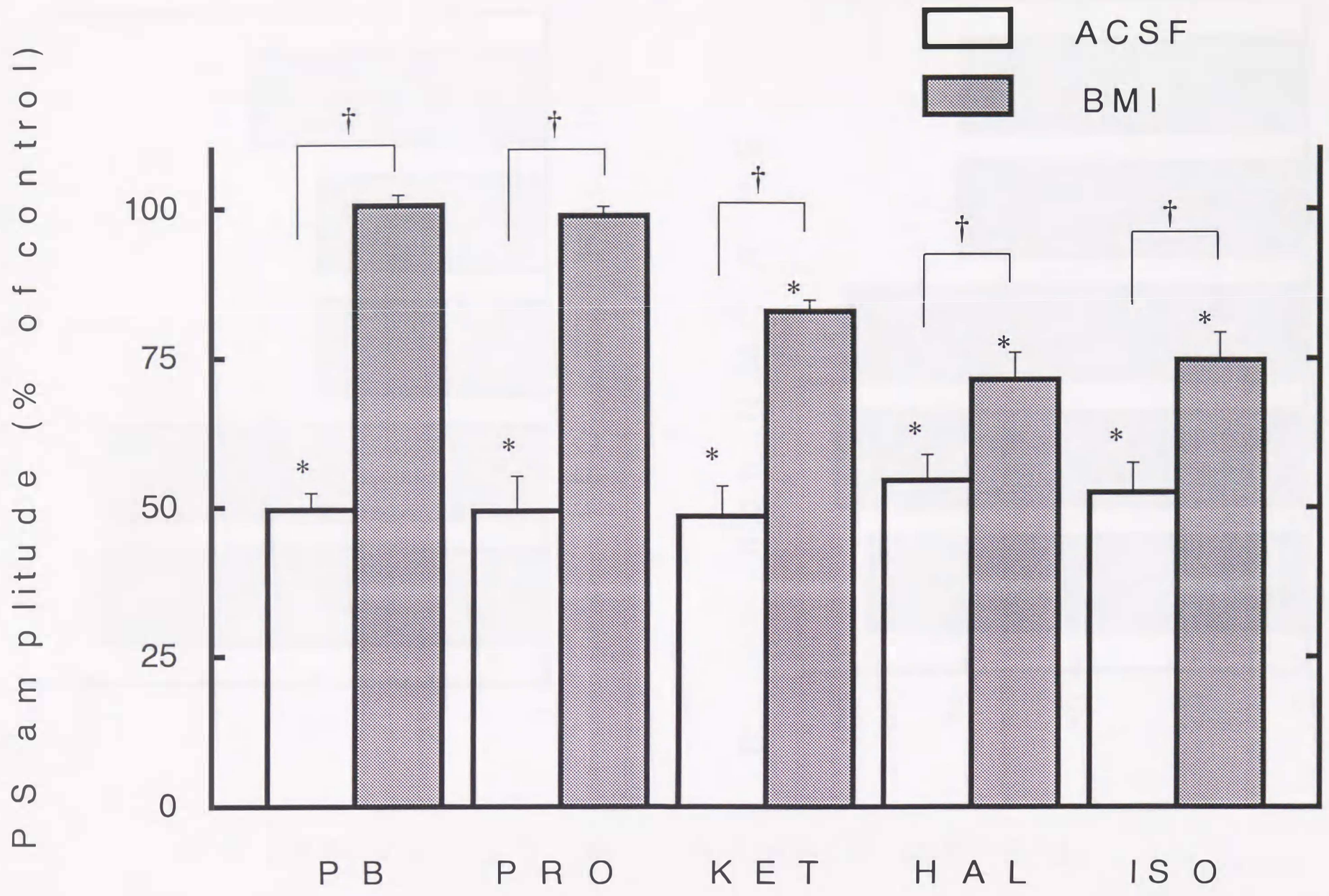
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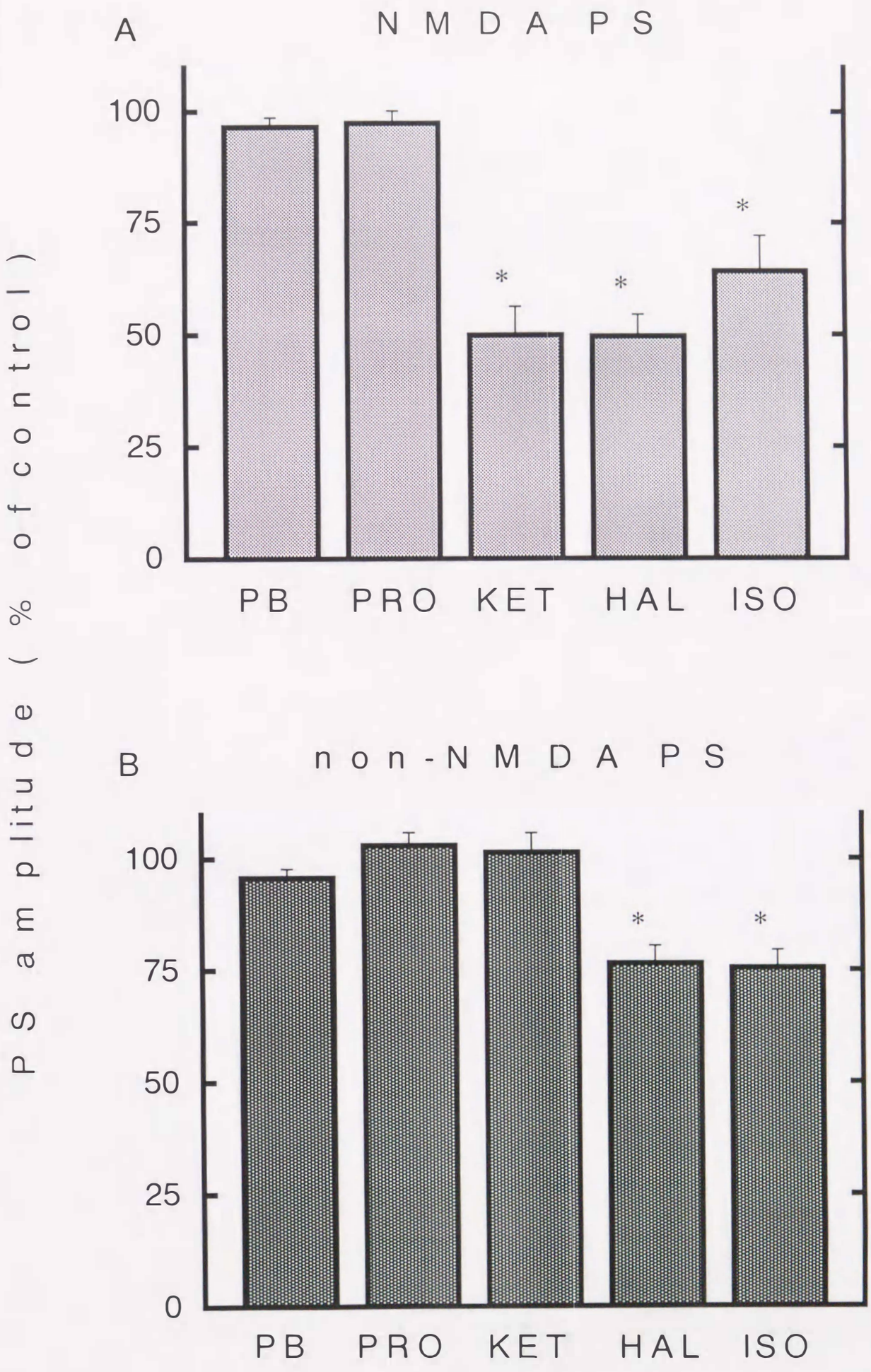
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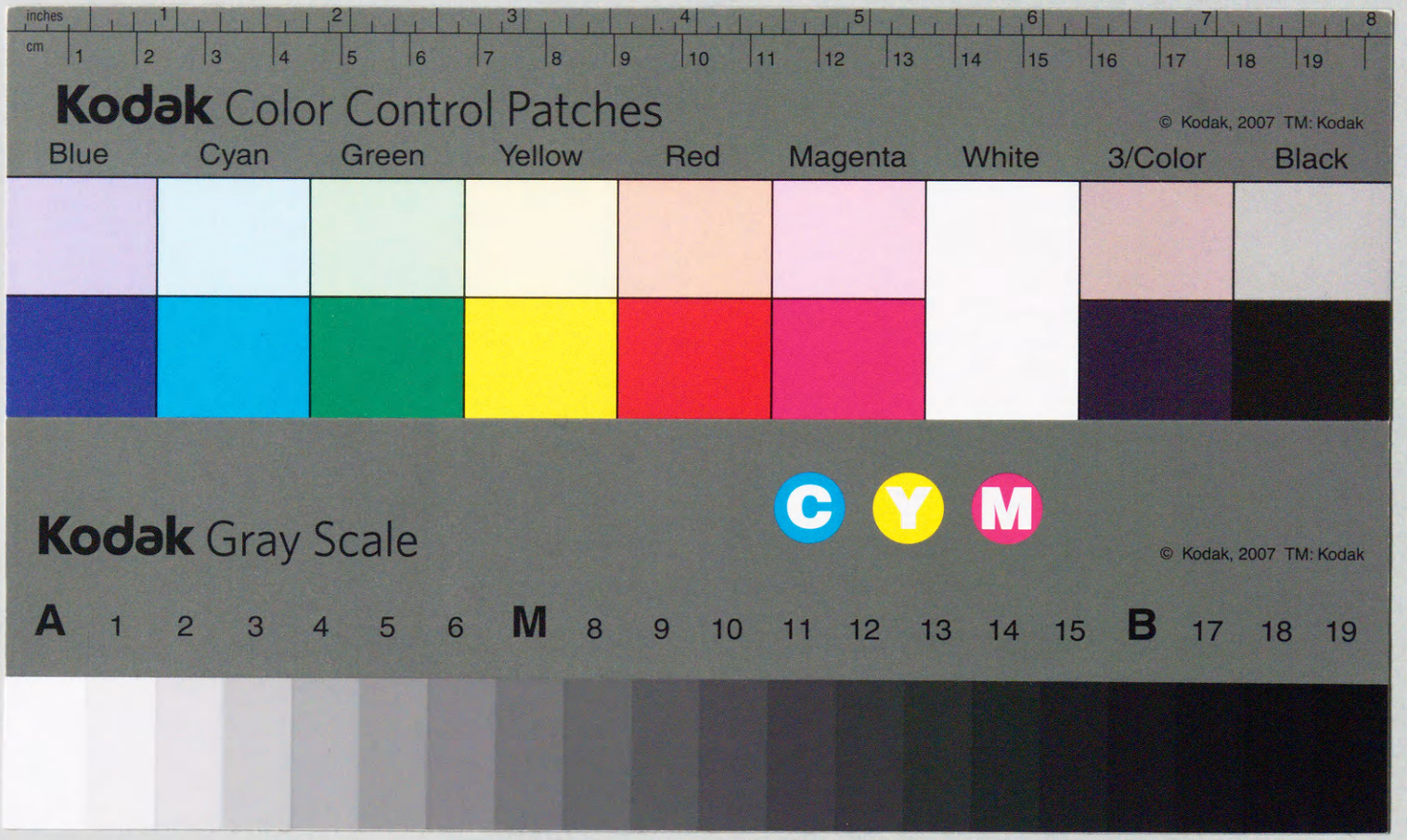
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