EPSP in single cells were observed by an intracellular microelectrode before and during an application of GABA. The amplitude of fast EPSP was gradually depressed with no detectable (Figure 1-B) or a slight depolarization (less than 3 mV) of the resting membrane under the effect of GABA. The effect of GABA on the acetylcholine (ACh) sensitivity of ganglion cells was studied by recording the nicotinic ACh depolarization produced by direct applications of ACh to the perfusate (Figure 1-C). No detectable changes of the amplitude of the ACh depolarization were observed under the effect of GABA.

The preganglionic nerve terminals were depolarized, whereas the preganglionic nerve axons were not affected, by the action of GABA ($1–10^{-2}$ m*M*) (Figure 2-A). Namely, in preganglionic nerve fibres, their terminal membrane was selectively depolarized by GABA. The depolarization of the preganglionic nerve terminal was partially restored (unlike the depolarization of ganglion cells), when GABA was applied for more than 1 min (Figure 2-A). It has been known that such a depolarization of preganglionic nerve terminals was produced by the action of nicotine[3]. The GABA depolarization disappeared during the nicotine depolarization of preganglionic nerve terminals (Figure 2-B). The GABA depolarization, however, could be produced after a transient nicotine depolarization subsided in the presence of nicotine (Figure 2-B). Furthermore, the GABA depolarization was produced in Ca-deficient (0.1 m*M* $CaCl_2$) Ringer's solution containing 6 m*M* Mg.

The amplitude of GABA depolarizations was decreased (or increased), while nerve terminals were depolarized (or hyperpolarized) by applying a constant cathodal (or anodal) current through a bridge-circuit[3]. This indicated that the GABA depolarization was produced by an increase of the membrane permeability to some ions. The GABA depolarization remained unchanged in the Na-free *Tris* solution.

The GABA depolarization could be inhibited in the presence of picrotoxin (Figure 2-C); when picrotoxin was applied to preparations, no depolarization of nerve terminals was observed. No effect of strychinine on the GABA depolarization was observed. Inhibitions of neither the nicotinic transmission nor the depolarization of presynaptic nerve terminals were observed in the presence of other amino-acids (less than 1 m*M*), such as L-glutamic acid, glycine or β-alanine.

Discussion. According to the present experiment, some kind of membrane receptor which is sensitive to GABA seems to be located at preganglionic nerve terminals. The main cause of the inhibition of the nicotinic transmission in the present preparation was apparently due to a reduction of ACh release from presynaptic nerve endings, being caused by the GABA depolarization at preganglionic nerve terminals.

The present results suggest that the GABA depolarization of preganglionic nerve terminals may be due to an increase in the membrane permeability to certain ions, presumably sodium and/or chloride ions. The fact that no appreciable changes in the GABA depolarization were observed in the Na-free *Tris* solution suggested that the GABA depolarization might be produced by an increase of the membrane permeability to chloride ions.

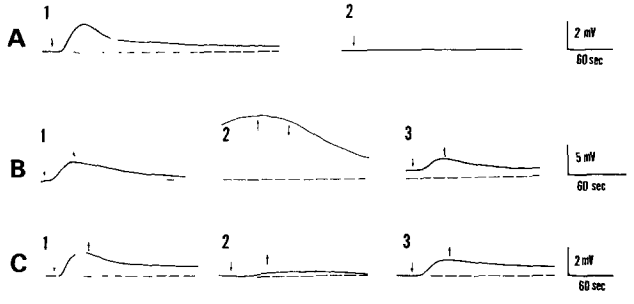

Fig. 2. Depolarizations of preganglionic nerve terminals produced by the action of GABA (0.1 m*M*). These records were taken by the sucrose-gap method. A) Record 1 and 2 were recorded from preganglionic nerve terminals and preganglionic nerve axons, respectively, when GABA was applied (shown by arrows). B) GABA depolarizations produced before (1), 2 min (2) and 10 min (3) after an application of nicotine (0.12 m*M*). GABA was applied for approximately 30–40 sec (shown by arrows). Note the original potential levels shown by broken lines. C) Effect of picrotoxin (0.01 m*M*) on the GABA depolarization. Record 1 and 2 were taken before and 20 min after an application of picrotoxin, respectively, and record 3 was taken 30 min after its withdrawal.

Zusammenfassung. Die hemmende Wirkung von GABA auf die synaptische Transmission wird analysiert und nachgewiesen, dass der Angriffspunkt des GABA offenbar präsynaptisch ist, indem es präsynaptisch zu deutlicher Depolarisation führt.

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Sodium Acetylsalicylate Effectiveness Against Fever Induced by Leukocytic Pyrogen and Prostaglandin E_1 in the Cat[1]

Small quantities of leukocytic pyrogen (LP) placed within the third ventricle[2] or directly into the preoptic/anterior hypothalamic area[3,4] are known to evoke a febrile response in the unanesthetized animal. Sodium acetylsalicylate (NaASA) has been shown to be effective as an antipyretic when administered at the same loci[2,5]. Since prostaglandin E_1 (PGE_1) has also proved to be a potent pyretic agent when discretely applied to the same region of the brain[6–8] and since the synthesis and release of endogenous PGE_1 are inhibited by NaASA[9], Vane has proposed that pyrogen fever may be mediated by PGE_1 in the preoptic/anterior hypothalamic area. The antipyretic action of NaASA against a controlled challenge of exogenous LP and PGE_1 has been utilized here to further examine the hypothesis that local synthesis and release of PGE_1 are implicated in the febrile response to leukocytic pyrogen.

Meterials and methods. Six healthy male cats weighing between 3.6 and 4.1 kg were used in this study. Under halothane anesthesia, cerebral cannulae were implanted stereotaxically to provide access to the third ventricle. Drug tests were begun 1 week after surgery and were conducted at weekly intervals thereafter. Body temperature was assessed with a rectal thermistor and telethermometer and continuously recorded on a polygraph. Normal body temperature was monitored for at least 1 h

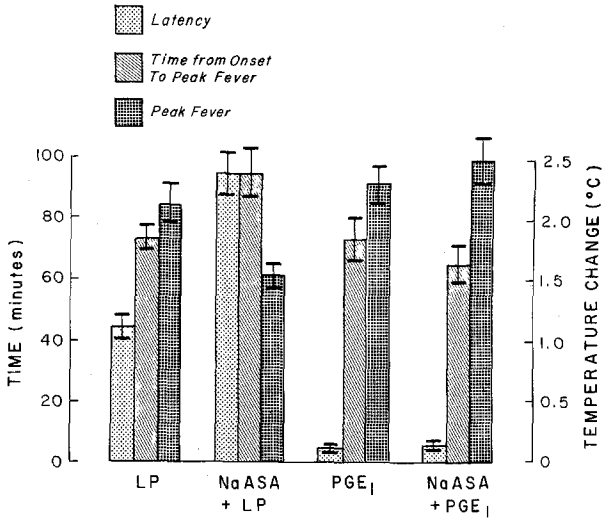
prior to drug administration. PGE_1[10] and NaASA[11] were solubilized in sterile 0.9% saline, adjusted to pH 7.3 and passed through a Millex filter. This vehicle was also used for control injections. Standard doses of cat leukocytic pyrogen[12] (0.1 ml) and PGE_1 (10 µg in 0.1 ml) were given via the 3rd ventricle. These doses were chosen for their comparable elevation of body temperature. NaASA (100 mg/kg i.v.) was injected into a brachial vein about 8 min before the pyretic agent was given. This dosage is known to inhibit or reduce fever due to leukocytic pyrogen[5].

Results and discussion. The febrile response was evaluated with the following parameters: a) latency in min from injection of LP or PGE_1 to the onset of fever, b) time in min from initiation of thermal rise to peak fever and c) the peak fever developed in degrees centigrade. In the absence of NaASA, the pyresis evoked by LP and PGE_1 placed in the 3rd ventricle were essentially equivalent in magnitude but developed over very different courses of time. A latent period of 44.2 ± S.E. 3.8 min was required before the increase of temperature began after injection of LP. On the other hand, the onset of fever with PGE_1 occurred only 4.3 ± 0.4 min after administration. In spite of this great disparity in latency ($p < 0.001$), it was found that the time to reach peak fever after onset was precisely the same for both. The average rise of body temperature obtained with the standard dose of LP was 2.12 ± 0.16 °C and that for PGE_1 was 2.29 ± 0.15 °C.

Pretreatment of the cats with NaASA demonstrably altered the response to LP but had very little effect on that to PGE_1. The latency after LP was dramatically prolonged from 44.2 ± 3.8 min to 95.0 ± 7.3 min by NaASA ($p < 0.001$), but that for PGE_1 was unchanged by the NaASA. The antipyretic agent also lengthened the period from onset to peak fever after injection of LP from 73.3 ± 3.8 min to 95.0 ± 8.4 min ($p < 0.1$), but it did not alter the duration of this period after PGE_1. The peak fever developed by the standard dose of LP was significantly reduced to an average rise of 1.52 ± 0.09 °C ($p < 0.02$). In contrast, the magnitude of the PGE_1 fever was essentially unaltered.

Chronometric evaluation of the febrile response has revealed some informative differences as well as similarities between LP and PGE_1 fevers of equal magnitude. The most significant difference was in the latent period. The average latency for LP alone was over 40 min while that for PGE_1 was only about 4 min. In addition, the most significant effect of NaASA was to delay the onset of fever after administration of LP. None of the antipyretic effects of NaASA exhibited against LP fever were apparent for the pyrexia due to PGE_1. This observation is consistent with that reported by MILTON and WENDLANDT[6] for the antipyretic compound acetamidophenol. The possibility that LP and PGE_1 may employ common mechanisms in producing fever may be inferred from their equivalent rates of fever development after onset. However, LP requires an initial rate-limiting, NaASA-sensitive process while PGE_1 does not.

The data obtained in this preliminary investigation are not inconsistent with the proposal[9] that LP may produce fever by stimulating the synthesis and release of PGE_1 in the preoptic/anterior hypothalamic area. If such were the case, then the long latency might reflect an induction period for PGE_1 synthesis. FELDBERG and GUPTA[13] have very recently reported evidence of a PGE_1-like substance in the cerebrospinal fluid of unanesthetized cats after endotoxin administration.



Evaluation of fever development in 6 cats after intracerebroventricular injection of leukocytic pyrogen (LP) and prostaglandin E_1 (PGE_1) alone and with sodium acetylsalicylate (NaASA) pretreatment.

Zusammenfassung. Fieber wurde durch die intraventrikuläre Injektion gleich wirksamer Dosen von Leukozyten-Pyrogen (LP) und Prostaglandin E_1 (PGE_1) hervorgerufen. Das Fieber entwickelte sich nach Beginn mit einer für beide Substanzen genau gleichen Anstiegsrate der Temperatur, die Latenzzeit bis zum Fieberbeginn war für LP jedoch länger als für PGE_1. Die i.v. Gabe von Natriumsalicylat verzögerte den Beginn, verlangsamte die Anstiegsgeschwindigkeit und setzte das Ausmass des LP-Fiebers herab, es war jedoch ohne Einfluss beim PGE_1-Fieber.

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[11] The NaASA was kindly donated by Bristol Laboratories, Syrasuce, N.Y.

[12] The cat LP was kindly prepared for us by Drs. C. Y. CHAI and M. T. LIN of the Department of Biophysics, National Defence Medical Center, Taipei Taiwan, Republic of China.

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