急性出血下電針刺激對血壓腎血流及腎交感神經之影響

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主持人: 林則彬

摘要

針刺(Acupuncture)在臨床醫學上已被廣泛的應用上千年，在之前的研究中已証實：電針刺激可以在正常鼠產生加壓之作用。本研究的主要目的則是在進一步探討電針刺激應用於急性出血之可行性，並釐清其神經機制。預定將以大白鼠為實驗動物，選定常用的兩處穴位：合谷(Li-4)及足三里穴(St-36)，分別以不同頻率(2 Hz、20 Hz，分別代表低頻、高頻)和刺激強度(20×T，T 為能引起肌肉收縮之最小電流閾值)相互組合分別給予刺激，測試其在急性出血時對大白鼠血壓、腎血流量及腎神經活性的影響。並測定何種刺激參數能夠引起最大的反應。再則，不同強度的電刺激能興奮不同大小種類的體神經，藉此亦可以推測引起電針效應的傳入神經纖維(afferent nerve)。若電針刺激可以在動物實驗獲得明顯的效應，便將進一步進行人體試驗，以評估電針刺激之臨床應用之可行性。

序論

背景及目的

針灸在中國用以治療各種疾病已有兩千多年的歷史，許多研究針灸生理機制的報告指出，針灸能透過體交感神經反射(somato-sympathetic reflex)影響身體各部的臟器功能(Andersson et al., 1973; Koizumi et al., 1980; Tsuchiya et al., 1991; Sato et al., 1993; Kimura et al., 1995)。

急性出血對人體的影響

在急性出血(acute hemorrhage)時，系統血壓下降，導致臟器之灌流血量減少。腎臟過度缺血將引發不可逆的病理變化，導致急性腎衰竭(acute renal failure)甚至死亡。

電針刺激對心臟血管系統的影響

目前已有許多針灸對心臟血管系統作用的報告： Lovic (1995)等人指出，以低頻的電流刺激大白鼠腓神經(peroneal nerve)能造成血壓上昇的現象。Sugiyama (1995)的報告亦指出針灸刺激足三里穴會使正常受試者的血壓上昇。

電針刺激的各個參數

但可惜的是，前述的各個實驗，雖然都同樣地使用針灸刺激引發加壓作用，但其作用的效用並未加以量化，在 Lin and Fu 的報告中証實：電針刺激正常大白鼠之合谷穴可以產生加壓之作用(Lin and Fu, 2000)。Lin and Fu 並將刺激的參數加以量化，並指出採用頻寬為 0.05 ms, 頻率為 2 Hz 與 20 Hz 的電針刺激，只要強度大於刺激閾值的五倍，便能夠引發系統血壓約 10% 的加壓反應。一旦刺激強度高於閾值的二十倍，系統血壓就會產生最大的反應，亦即血壓增加約 15%。

Lin and Fu 進一步的人體實驗指出：相同參數的刺激亦可提高人體之血壓及指端之血流量(unpublished data)。顯示電針刺激的效應具有臨床應用的價值。

因此本研究以動物實驗的模式，先行評估電針刺激在急性出血狀況下之作用，以作為將來進一步人體實驗之參考。除了仍採用電針刺激的方式將刺激參數量化外，亦將測試兩種不同頻率的電針刺激，與各個不同強度作組合，觀察其所誘發的反應，以鑑別出何種型式的電針刺激最具功效。

材料與方法

外科手術

本實驗以大白鼠為實驗動物，先施以氣體麻醉(penthrane)，在手術後再利用阿爾發氯糖(alpha-chloralose)以靜脈注射維持麻醉。在整個實驗的過程中定期地觀察動物的呼吸，血壓，心律及自發性動作，以判別麻醉深度是否得宜，決定是否追加麻醉劑量。

動物的左、右股動脈及右股靜脈分別加以插管，分別作為測量血壓，失血及注射藥品之用。氣管亦加以插管，以備必要時進行人工呼吸。

動物以仰臥的姿勢進行手術，在上腹腔施行正中切開。交感神經到達腎臟的分枝與週圍組織以手術器材加以分離，並以下列方法加以檢定。其一是將神經以電刺激加以檢定。若電刺激(20 Hz, 5 mA, for 5 sec)引起腎臟發生漂白反應(bleaching reaction)，則可確定為腎神經。另外則自右股動脈抽出約 1 c.c 血液，若腎神經自發性放電增加(> 110% of control) 亦可確定為腎神經。

切開的腹腔則覆以溫石蠟油以避免乾燥。動物的肛溫則以紅外燈維

持於攝氏 37 度左右。

電針刺激

刺激穴位參照中國傳統的針灸圖譜，以解剖學的相對位置在動物體上加以定位。足三里穴位於脛骨的上緣，合谷穴則位於上肢第一及第二掌骨交接處。以一隻焊接於電線的皮下針垂直插入刺激點，另一隻相同的針則插入相距約 5-10 mm 處作為正極。

電流則以刺激器(Grass，S88)輸出通至絕緣器(Grass，SIU5B)再以穩流器(Grass，CCU1A)輸出至動物體。刺激的頻率則分兩組，低頻電針刺激組為 2 Hz，此乃一常見的捻針頻率。高頻刺激組則為 20 Hz，相當於低頻刺激的十倍，用以比較高低頻刺激之差異。

每一頻率分別以閾值 20 倍的刺激強度(20×T) 加以刺激測試。所有的測試其刺激時間皆設定為十分鐘，因為本實驗所引發的反應在此時間內已達飽和。

記錄

血壓以壓力轉換器(Statham，P23D)經由插入動脈的導管予以連續記錄在多項記儀(Gould，2200S)。腎血流量則以電磁血流轉換器(electromagnetic flow probe)環繞於腎動脈之上，並將訊號傳至流量計中加以放大及記錄。

腎神經的活性則以一對不鏽鋼絲電極加以記錄，經由交流前置放大器(Grass，P511AC)顯示於示波器上。神經的活性以視窗選擇器(WPI，

WP5)加以選擇後，腎神經放電的數目則以計數器(Gould，13-4615-70)每五秒計數一次，並記錄於多項記錄儀(Gould，2200S)。

急性出血

以麻醉狀況下之大白鼠血壓作為控制組，先進行第一次電刺激之實驗。待實驗完成後，以肝素潤濕過之注射針筒自股動脈抽取約 2-3 ml 之血液，此時動物之血壓先迅速下降，接著便因壓力反射(baroreflex)之調控，在三分鐘內逐漸恢復至約為控制血壓的 90%。再進行第二次電刺激實驗。待實驗完成後，再抽取約 3-4 ml 之血液，三分鐘後(血壓約恢復至控制值的 75%)再進行第三次實驗。

數據統計

本實驗以 *t*-test 統計檢測組間的差異，P 值小於 0.05 為最小的顯著差異值。

結果

圖一

此圖是電針刺激合骨穴對血壓和腎神經的影響。在 intact rats 身上我們可以看到電針刺激合骨穴不論是 2Hz 或 20Hz，血壓與腎神經均為同步變化。不同電針刺激頻率會引發不同反應，2Hz 為 tonic，20Hz 為 phasic。電針刺激足三里穴不論 2Hz 或 20Hz 均無法引發出 pressor effect。

圖二

失血初期，電針刺激所引發的血壓與腎神經的 pressor response 變

化。在輕微失血的 group 中，電針刺激可使血壓與腎神經活性平行地回升至 control level，而其他 H2、H3 group，不論 2Hz 或 20Hz，電針刺激可使 RNA 呈現 pressor effect 但血壓加壓效果卻明顯減弱。

圖三

統計圖中圓圈代表 intact、正三角形代表 H1、方形代表 H2、倒三角形表 H3。電針刺激所引發的腎神經活性 peak pressor effect 卻顯示出 intact、H1、H2、H3 group 之間沒顯著差異，這意味著它們引發反應的神經路徑可能有 overlap。

討論

血壓的加壓效果在輕微失血可以被引發出，但在嚴重失血下則效果不好，神經的加壓反應不論在正常或失血下，效果都很明顯。且藉由電針刺激所引發的加壓反應與失血具有共同神經路徑，即是 baroreflex。在臨床應用方面，我們可用在正常或輕微失血的病人身上，但對低血量的病人則有所限制。

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Pressor effect on blood pressure and renal nerve activity elicited by electroacupuncture in intact and acute hemorrhage rats

Hua Ting[a], Jiuan-Miaw Liao[b], Chih-Feng Lin[c], Ping-Yen Chiang[c], Chi-Chen Chang[b], Dong-Yih Kuo[b], Tzer-Bin Lin[b,*]

[a]*Chung-Shan Medical University Hospital, Taichung, Taiwan*

[b]*Department of Physiology, College of Medicine, Chung-Shan Medical University, No. 110, Chang-Kuo North Road, the first Section, Taichung 10018, Taiwan*

[c]*School of Physical Therapy, Chung-Shan Medical University, Taichung, Taiwan*

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Abstract

The neural mechanism underlying the effect of electroacupuncture (Ea) on arterial blood pressure (BP) and renal nerve activity (RNA) in the intact state and during acute hemorrhage was investigated in anesthetized rats. Two acupoints, Hoku (Li-4, at the junction of the first and the second metacarpal bone) and Tsusanli (St-36, at the lateral upper tibia bone), were tested using Ea of two different frequencies (2 and 20 Hz). In the intact state, Ea at Hoku elicited an elevation of BP in parallel with RNA, while Ea found no response with identical parameters at Tsusanli. The pattern of the pressor response caused by a low frequency Ea (2 Hz) at Hoku was different than a high frequency one (20 Hz), i.e. a tonic effect was elicited with 2 Hz, while a phasic one was induced with 20 Hz. In mild hemorrhage conditions (10% of BP decrease), similar pressor effects, as in intact rats, were also elicited by Ea. However, in severe hemorrhage conditions (20 and 30% BP decrease), Ea induced a pressor effect on RNA and an attenuated effect on BP. BP and RNA showed a significant correlation in intact and mild hemorrhage conditions, but not in severe hemorrhage conditions. All the results suggested that Ea at Hoku with appropriate stimulation parameters can increase and maintain BP in normal and hemorrhage conditions, and such a therapeutic technique has potential in clinical practice.

Keywords: Electroacupuncture; Renal nerve; Blood pressure; Hemorrhage; Rat

Acupuncture has been used in China to treat various diseases for more than two thousand years. Researchers exploring the physiological mechanism underlying acupuncture suggested that acupuncture may modulate nerve activity influencing visceral function via somato-sympathetic reflexes [1,8,9,15,18]. Many investigations were concentrated on the effect of acupuncture on the cardiovascular system [10,20,21]. Acupuncture stimulation was reported to elicit a pressor effect on human subjects [17] and anesthetized rats [14].

The mechanism that underlies electroacupuncture (Ea) to induce a pressor effect on blood pressure (BP) was investigated by Lin and Fu [12,13]. In their study, a pressor effect was elicited by potentiation of sympathetic tone. Sato et al. [15] and Liao et al. [11] investigated the therapeutic effect induced by Ea on a hyperactive stomach and suggested that segmental sympathetic outflow was activated by Ea to induce a therapeutic effect.

When a person bleeds severely so that the BP falls suddenly, the sympathetic outflow is excited immediately to return the arterial pressure and maintains a high enough level so that the person can live through the episode. Since sympathetic excitation is the main route in an acute hemorrhage condition to restore adequate BP, the possibility of Ea being employed in the treatment of acute hemorrhage seems to be fascinating. Therefore, in this study, Ea was used to test whether a pressor effect can be induced in intact and hemorrhage conditions. In order to analyze stimulation and strength both qualitatively and quantitatively, an electric current was used in this experiment to stimulate acupoints [11–14].

Sato and Schmidt [16] reported complex visceral responses to acupuncture stimulation in animal experiments, indicating that the site of acupuncture was one of the important factors causing different responses in visceral

* Corresponding author. Tel.: +886-4-2473-0022-1652; fax: +886-4-2473-9030.

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organs. To test whether Ea with identical stimulation parameters at different sites may induce different response or not, two acupoints, Tsusanli (St-36) and Hoku (Li-4), were tested in this study. These two acupoints have been widely used in a number of investigations and were easily accessible [2,4–7,19]. Therefore, effects of Ea with various stimulation parameters at Tsusanli and Hoku on BP and sympathetic nerve activity in anesthetized rats were investigated in this study. For this purpose, we selected sympathetic adrenal nerve activity (RNA) as a representative index of vasoconstrictive nerve activity [14].

Twenty adult female SD rats, weighting 220–350 g, were used throughout this study. Animals were anesthetized initially with penthrane (methoxyflurane, by Abbott Lab.) for surgical preparations, and then were maintained under alpha-chloralose anesthesia (50 mg/kg, i.v.). Bilateral femoral arteries were cannulated for BP recording and hemorrhage, and the right femoral vein and trachea were also used for anesthetic administration and maintaining of airway potency. Systemic BP was continuously recorded on a computer system (Biopac MP30) through a transducer (Statham P23) with an arterial catheter. The abdomen was opened by a median incision; branches of sympathetic nerve to the left kidney were dissected free from surrounding tissue and identified by a bleaching reaction in response to electrical stimulation (20 Hz, 0.5 mA, for 5 s). The opened abdominal cavity was covered with warm paraffin oil to avoid drying. The rectal temperature was maintained at around 37 °C using an infrared lamp.

Acupoints were determined by transposing anatomically from Chinese traditional human acupuncture charts. Two acupoints, Tsusanli, located at the lateral upper tibia, and Hoku, located at the junction of the first and the second metacarpal bones, were tested. An interdermal needle (32 gauge, 1/2 inches long, by Trueline Instruments) soldered to a flexible electrical wire was inserted vertically into the selected acupoints. A second identical needle, as a positive pole, was inserted into the other point approximately 5–10 mm from the first one. An electric current of square wave pulses with a pulse duration of 0.05 ms was applied from a stimulator (Grass S88) through a stimulus isolation unit (Grass SIU5B) and a constant current unit (Grass CCU1A). Two stimulation frequencies, 2 and 20 Hz, were tested in this experiment. A frequency of 2 Hz was widely employed in manual and electric acupuncture studies, and 20 Hz was ten times the former and served as a high frequency stimulation. The stimulation intensity was 20 times the threshold (the minimal stimulation intensity to induce muscle twitch). The total stimulation time in this study was set for 10 min because the effect of Ea on BP and RNA became stable within 5 min in this study.

Renal efferent nerve activities were recorded after crushing the nerve as close as possible to the kidney [3]. Nerve activities were recorded with a pair of stainless steel wire electrodes through an AC preamplifier (Grass P511AC) and displayed on an oscilloscope (Gould DSO 1604). Through a window discriminator (WPI WP5), the firing frequency of the adrenal nerve was counted by a spike counter (Gould 13-4615-70) every 5 s and recorded on a polygraph (Gould 2200S).

Blood was slowly withdrawn through the arterial catheter to induce hemorrhage. The decrease in BP was 10, 20 and 30% to the control value (H1, H2 and H3 groups, respectively). In the H3 group, the BP was difficult to maintain at this level, and a gradual recovery was commonly seen after a hemorrhage procedure.

Statistical differences between groups were determined using two-way ANOVA followed by Student's t-test. $P < 0.05$ was accepted as a minimal level of significance.

In intact rats, BP and RNA were raised in parallel by Ea at Hoku, both with 2 and 20 Hz frequency. However, the patterns of response elicited by these two different frequencies of Ea were distinct from each other (Fig. 1).

BP and RNA in response to Ea with a frequency of 2 Hz are shown in Fig. 1A. Following the onset of Ea, a pressor response was slowly induced and a maximum effect was reached within 30 s (110–120 and 150–200% of control in BP and RNA, respectively). Then, BP and RNA were maintained at this level until cessation of stimulation. The effect of Ea with a frequency of 2 Hz on BP and RNA under an intact condition is summarized in Fig. 3A (circle).

BP and RNA in response to Ea with a frequency of 20 Hz are shown in Fig. 1B. A sharp pressor response was induced following the onset of Ea. A peak effect was reached within 10–15 s (140–170 and 250–320% of control in BP and RNA, respectively). Then, BP and RNA were gradually returned to the control level in the subsequent 2–3 min.

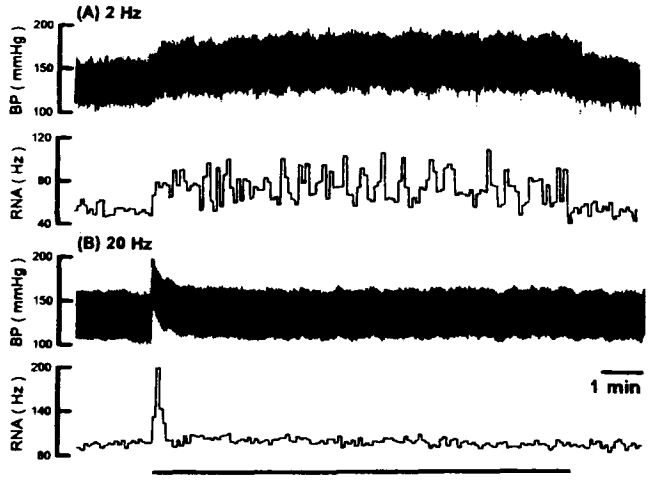

Fig. 1. Effect of Ea on BP and RNA. Note that Ea with a frequency of 2 Hz (A) and 20 Hz (B) elicited a pressor effect on BP and RNA. The black bar at the bottom represents Ea for 10 min. (A) Following the onset of Ea, a pressor response was slowly induced and a maximum effect was reached within 30 s, and then maintained at this level until the cessation of stimulation. (B) A sharp pressor response was induced following the onset of Ea, and a peak effect was reached within 10–15 s, and then was gradually returned to the control level in the subsequent 2–3 min.

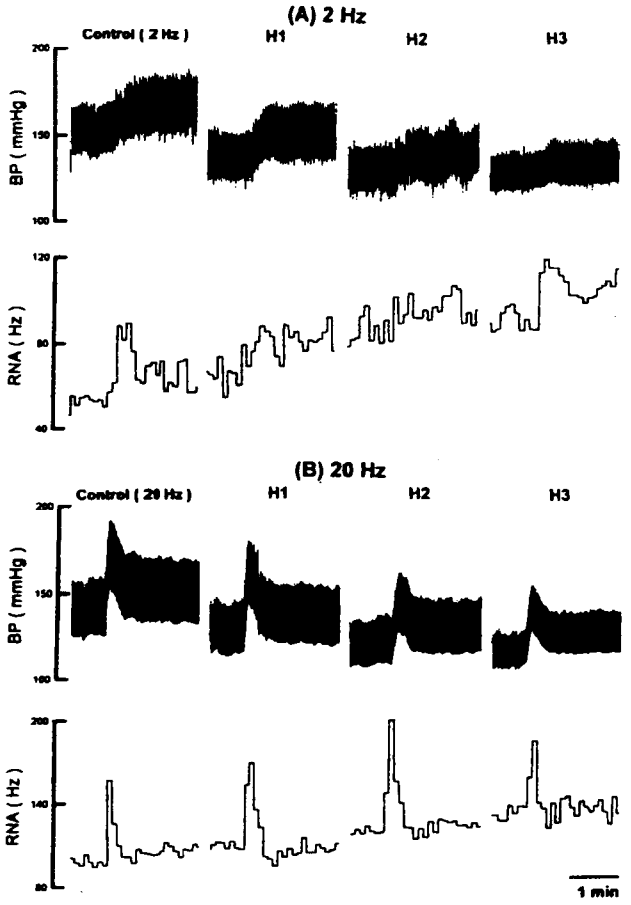

Fig. 2. Pressor response on BP and RNA elicited by Ea under hemorrhage conditions. BP and RNA were raised in parallel by Ea in intact and H1 groups; however, in H2 and H3 groups, the pressor effect on BP was attenuated while that on RNA remained unchanged.

The effect of Ea with a frequency of 20 Hz on BP and RNA under an intact condition is summarized in Fig. 3B (circle).

At Tsusanli, neither Ea with a frequency of 2 Hz nor that of 20 Hz induced a pressor effect on BP and RNA.

The early period of the pressor response on BP and RNA elicited by Ea with a frequency of 2 and 20 Hz under hemorrhagic conditions is shown in Fig. 2A,B, respectively. In the H1 group, BP and RNA were raised in parallel by Ea. However, in the H2 and H3 groups, the pressor effect on BP was reduced while that on RNA remained unchanged. The effect of Ea with a frequency of 2 Hz on hemorrhage conditions is summarized in Fig. 3A,B, respectively.

The BP–RNA relationship during the early pressor response (within 20 s after Ea onset) was further investigated. Since the results from 2 and 20 Hz Ea showed no statistical difference, they were pooled together. As shown in Fig. 4, the correlation between BP and RNA during the early pressor response was significant in intact and H1 groups ($r = 0.29$ and 0.33, respectively, $n = 10$), while no significance was shown in H2 and H3 groups.

The present study demonstrated that Ea at Hoku elevated

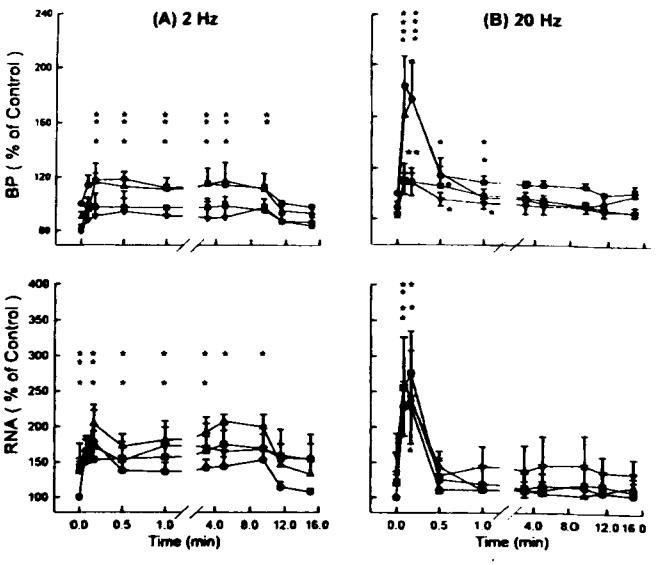

Fig. 3. Effect of Ea on BP and RNA under intact and hemorrhage conditions. Circle, intact; triangle, H1; square, H2; reversed triangle, H3. The pressor response on BP elicited by Ea was attenuated in the H2 and H3 groups, while that on RNA remained unchanged. Note that despite the basal firing of RNA being elevated by hemorrhage, the peak pressor effect on RNA caused by Ea showed no significant difference among intact, H1, H2 and H3 groups ($^*P < 0.05$, $^{**}P < 0.01$, $n = 10$).

BP and RNA in intact rats, but stimulation with identical parameters at Tsusanli caused no effect. The pressor effect on BP caused by Ea was also elicited in mild (H1 group) but attenuated in severe (H2 and H3 groups) hemorrhage conditions.

The result that Ea at Hoku induced a pressor effect on BP was correlated with the report of Ernst and Lee [5], who found a transient segmental increase in sympathetic nerve activity induced by electrical and manual acupuncture

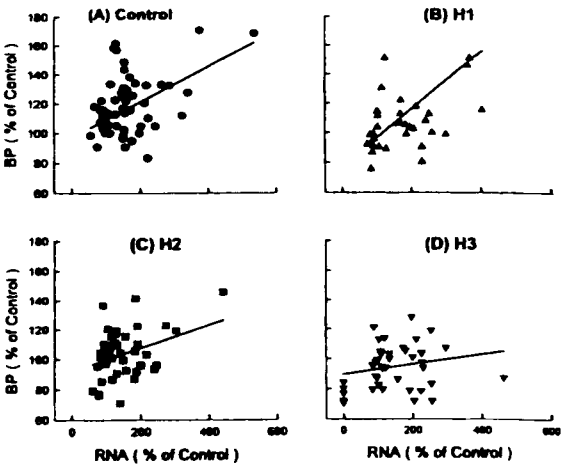

Fig. 4. BP–RNA relationship in the early pressor effect under intact (A) and hemorrhage (B–D) conditions. The correlation between BP and RNA was significant in intact and H1 groups ($r = 0.29$ and 0.33, respectively, $n = 10$), but no significance was shown in H2 and H3 groups.

stimuli at Hoku in normal subjects. On the other hand, BP of normal subjects was not affected during manual acupuncture at Tsusanli, which was also reported by Sugiyama et al. [17].

As shown in Fig. 3, a parallel increase was found in BP with RNA during Ea at Hoku in intact and mild hemorrhage conditions. RNA is thought to be an index of sympathetic tone, i.e. sympathetic outflow was excited by Ea and induced such a pressor response. This result is supported by Fig. 4A, which shows that BP and RNA showed a significant correlation in intact and mild hemorrhagic conditions. Furthermore, the pressor effect on BP elicited by Ea was mainly due to the potentiation of the sympathetic tone, which was also suggested by Lin and Fu [14]. On the other hand, in the severe hemorrhage group, Ea with an identical stimulation parameter elicited a pressor effect on RNA, but the effect on BP was attenuated (Fig. 2). Regarding Fig. 3C,D, the correlation of BP and RNA was not significant in severe hemorrhage conditions. In our conjecture, the volume lost in severe hemorrhage conditions is so much that, despite the sympathetic tone triggered by Ea and elevated RNA, BP failed to arise because the circulatory volume is low. As shown in the lower panel of Fig. 3, the basal firing of RNA was elevated gradually by hemorrhage but the peak pressor effect caused by Ea showed no significant difference among intact, H1, H2 and H3 groups. This result indicating the pressor effect elicited by Ea on BP shares, at least a part of, a common efferent pathway with baroreflex.

The precise neural mechanism involved in the pressor effect elicited by Ea in these conditions needs further investigation. Despite there being some limitations in hypovolumic patients, with appropriate stimulation parameters, the effect of Ea at Hoku increasing and maintaining BP in intact and mild hemorrhage conditions has potential in clinical practice.

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