KAWASAKI MED. J. VOL. 8 : NO. 3-4, 179-186 (1982)

Convergence of Cutaneous Afferent Impulses on Units of the Pontine Defecation Reflex Center in the Dog

Hiroyuki FUKUDA and Kiyoko FUKAI

Department of Physiology, Kawasaki Medical School, Kurashiki 701-01, Japan
Accepted for Publication on August 24, 1982

ABSTRACT. The convergence of cutaneous afferents was studied in 168 pontine reticular units. The units responded to afferent stimulation of rectal branches of the contralateral pelvic nerve of the dog, and were, therefore, regarded as candidate cells for the pontine defecation reflex center. About one third of the candidate units responded to cutaneous stroking, although most responded to cutaneous stimulation in the noxious range. The candidate units activated by both stroking and noxious stimulation were 21 of 65 units tested. Three units were excited by stroking although not by the noxious stimulation. Twenty-two of the 54 candidate units of which the spontaneous discharges were inhibited by afferent stimulation of the contralateral rectal branches were excited by the noxious stimulation. Most candidate units responded to cutaneous stimulation of two or more of the seven body parts tested. Latency of the responses to tapping and electrical stimulation of both hindlegs did not significantly differ from that of responses to the hunting stimulation. These results confirm that the pontine defecation reflex center mediates the effects of cutaneous stimulation on outflow of the rectal branch and on colonic motility.

Some pontine reticular units of dogs receive afferent impulses from the colon through the pelvic nerve. Some have discharge spikes that precede reflex discharge of the nerve, followed by propulsive contractions of the distal colon, and initiation of defecation[1]. From this and other findings in experiments of transection[2,3,4], localized lesion[3] and stimulation[5] of the brain stem, Okada et al. postulated that the pontine defecation reflex center exists in the pontine reticular formation.

On the other hand, Okada and Fukai (unpublished results) found that outflow in the rectal branch of the pelvic nerve and rectal motility were inhibited or enhanced by noxious or innoxious cutaneous stimulation, respectively. These effects disappeared after transection of the caudal pons. This suggests that cutaneous afferents converge on cells forming the pontine defecation reflex center. This suggestion was examined in this work.

福田博之，深井喜代子

METHODS

Experiments were performed on 25 dogs weighing 6–12 kg. Fourteen dogs were anesthetized with intravenous α-chloralose (80–100 mg/kg). Other 11 dogs were decerebrated precollicularly under electrical narcosis induced by application of an alternating current bitemporally (60 Hz, 60 volts and 15 sec duration). The animals were fixed on a stereotaxic headholder, paralyzed with gallamine triethiodide (1 mg/kg) then ventilated artificially through a tracheal cannula at a rate of 20–25 strokes/min and a tidal volume of 150–250 ml. Arterial blood pressure was monitored through a cannula connected to the femoral artery. Body temperature was maintained at about 36° by the light from two 100-watt tungsten lamps.

The ventral surface of the pons and the rostral medulla was exposed by a cranectomy. Rectal branches of the bilateral pelvic nerve were exposed by a mid-line incision. The lumbar colonic nerve and the bilateral hypogastric nerves were divided in all dogs. A small strand of the left rectal branch was prepared for recording of efferent activity. Central cut-ends of the right rectal branches were stimulated as the hunting stimulation for pontine reticular units. Bipolar electrodes of platinum wire were used for stimulation and recording. A silver wire electrode insulated with a glass micropipette was used for unit recordings[1]. The electrode tip was 4–8 μm in diameter and about 10 μm in length. The electrode was inserted ventrodorsally from the ventral surface of the pons by a hydraulic micromanipulator mounted on the stereotaxic headholder. Unit responses to the hunting stimulation were explored within the left half of the pontine reticular formation from 2.0 to 3.5 mm lateral to the mid-line. The pontine reticular units responding to the hunting stimulation were provisionally regarded as the candidate units for the cells forming the pontine defecation reflex center.

Effects of stroking, tapping and electrical stimulation of the skin were tested on the candidate units. Central foot pads of the four legs were stimulated with a single pulse of 40 mA and 1 msec duration by bipolar needle electrodes that were two injection needles fixed in parallel with an intervening space of about 5 mm. Flanks, forelegs, hindlegs and tail were stroked with fingers, and were tapped with a push button of a micro-switch, whose signal triggered an oscilloscope sweep. Both the tapping and electrical stimulation are in the noxious range, because they caused us pain, although the stroking did not.

The activity of the candidate unit was amplified with a high input resistance amplifier and RC coupled amplifiers, and then fed to a spike counter in parallel with an oscilloscope. The counted outputs were recorded with a pen recorder. The efferent activity of the left rectal branch was amplified with RC coupled amplifiers. Both of these activities were displayed on the oscilloscope and recorded directly on film with a long recording camera.

RESULTS

In our previous paper[1], the pontine reticular units regarded as the candidate units for cells forming the pontine defecation reflex center were classified into four groups by following four types of sequentially firing spikes in the response to the hunting stimulation : 1. a short burst with a short latency ; 2. an early short burst followed by a late long lasting discharge ; 3. a long lasting discharge with a long latency and 4. a temporal inhibition of spontaneous discharges. Group I, II, III and IV units responded with type 1, 2, 3 and 4, respectively. This classification will be used in the presentation of the present results.

Response of the candidate unit to stroking of the skin

The responding properties of the candidate units to cutaneous stimulation under the decerebrated condition were similar to those during chloralose anesthesia, therefore the results obtained under both conditions will be presented together.

The effects of cutaneous stroking were tested on 115 candidate units. Fig. 1, A and B show two examples of the test. The discharges of a group I unit were enhanced by stroking of the contralateral foreleg and bilateral flanks (Fig. 1, A), although spontaneous activity of a group IV unit was inhibited by stroking of the contralateral foreleg, flank and hindleg (Fig. 1, B).

The responses of 115 candidate units are summarized in Table 1. Cutaneous stroking accelerated the discharge frequency in 29 of 78 candidate units that were excited by the hunting stimulation (group I, II and III), although the spontaneous discharges of four of the 78 units were transiently inhibited. The inhibition occurred in eight of 37 units of group IV that were inhibited by the hunting stimulation. The receptive fields of 36 of 43 units that responded to the stroking were wider than one stroking part and the 14 units responded to stroking of all seven parts tested.

The responses of the candidate unit to tapping and electrical stimulation

Fig. 1, C and D show the responses of a candidate unit of group I to cutaneous tapping and electrical stimulation. This unit responded with an early short burst to both types of stimulation applied on all four-legs. As shown, the responding properties of the candidate units to tapping were similar to those of electrical stimulation, therefore both responses will be presented together.

The responses of 168 candidate units to both noxious stimuli are summarized in Table 2. The units responding to the noxious stimulation were 71% of 168 candidate units. This percentage was higher than that (37%) of units responding to the stroking. Like the responses to the hunting stimulation, most units (78%, 90%) of group I and II responded to the noxious stimulation with an early short burst, and late discharges were mainly observed in the responses from group II and III units. These results show that the properties of the responses of most units of group I, II and III to the noxious stimulation did not differ fundamentally from those to the hunting stimulation. Twenty-two

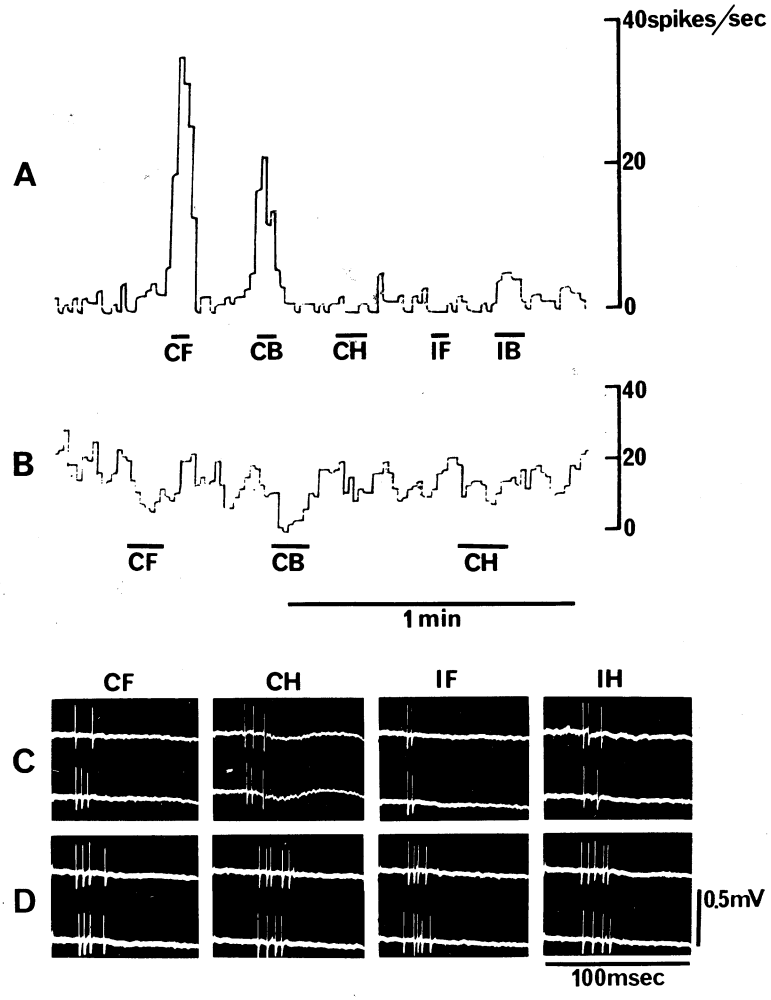

Fig. 1. Responses of the candidate units to cutaneous stimulation.
A ; a group I unit. B ; a group IV unit. During horizontal bars, the cutaneous parts indicated by abbreviations were stroked in A and B. CF ; contralateral foreleg. CB ; contralateral flank. CH ; contralateral hindleg. IB ; ipsilateral flank. C and D ; the same unit of group I. Four legs indicated abbreviations were tapped in C and their central foot pads were stimulated with single pulse of 40 mA and 1 msec duration in D. Tapping or electrical stimulation was applied at the starting point of each sweep. Responses to two sequential stimulation are shown on each photograph. IF ; ipsilateral foreleg. IH ; ipsilateral hindleg.

(40%) of 54 units of group IV, however, discharged after the noxious stimulation and only twelve units responded with inhibition of spontaneous discharges as in the responses to the hunting stimulation. All but three of the candidate units responded to the noxious stimulation of two or more of the seven body

TABLE 1. Responses of the candidate units to the stroking of the skin. Unit numbers (%) are shown.

Response types \ Unit groups	I	II	III	IV	Total
Enhancement	10 (37)	14 (48)	5 (23)	2 (5)	31 (27)
Inhibition	0 (0)	2 (7)	2 (9)	8 (22)	12 (10)
No change	17 (63)	13 (45)	15 (68)	27 (73)	72 (63)
Total	27(100)	29(100)	22(100)	37(100)	115(100)

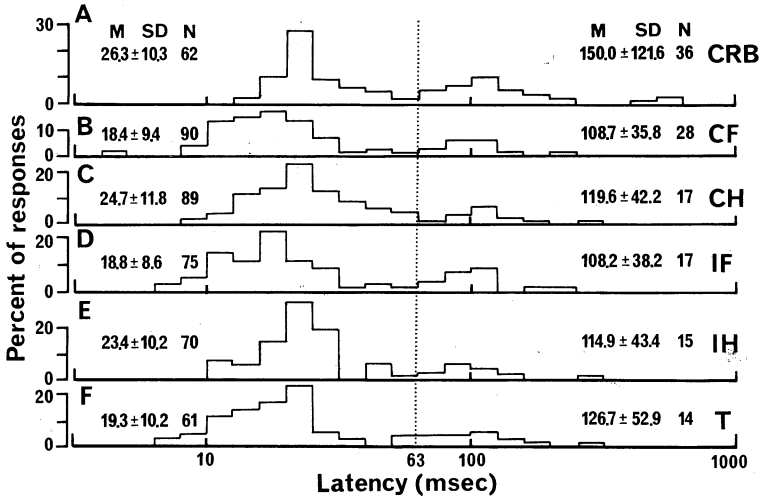

Fig. 2. Initial spike latencies of responses of the candidate units to the hunting and the cutaneous stimulation.

Histograms show initial spike latencies in responses to the hunting (A), contralateral foreleg (B), contralateral hindleg (C), ipsilateral foreleg (D), ipsilateral hindleg (E) and tail (F). Responses of the candidate units to these stimulation were divided at 63 msec into the fast and late groups. Numbers (N) and mean latency (M) with standard deviation (SD) of the fast group responses are shown on the left side of each histogram, and those of the late group responses are shown on the right side.

parts tested.

Receptive fields for the stroking were compared with those of the tapping in 32 candidate units. Eight units had the same receptive fields for both stimuli, 20 units had wider receptive fields for tapping than for stroking and only four units had narrower ones for tapping than for stroking.

The responses for both tapping and stroking were compared in 65 candidate units. Thirty-three units (51%) responded to tapping only, 28 units (43%) responded to both stimuli whereas three units (5%) (one of group II and two of group III) were enhanced only by, and one group IV unit was inhibited only by, stroking. All but three of the units were either inhibited or enhanced by

TABLE 2. Responses of the candidate units to the tapping and electrical stimulation of the skin. Unit numbers (%) are shown.

Response types \ Unit groups	I	II	III	IV	Total
Early short burst	39 (76)	20 (58)	5 (20)	13 (24)	77 (46)
Early short burst and late discharge	1 (2)	12 (32)	0 (0)	3 (6)	16 (9)
Late discharge	0 (0)	0 (0)	4 (16)	0 (0)	4 (2)
Inhibition	0 (0)	0 (0)	0 (0)	12 (22)	12 (7)
Discharges followed by an inhibition	1 (2)	4 (10)	0 (0)	6 (11)	11 (7)
No response	10 (20)	2 (5)	16 (64)	20 (37)	48 (29)
Total	51(100)	38(100)	25(100)	54(100)	168(100)

both stroking and tapping. The three exceptional units of group II were enhanced by tapping, although they were inhibited by stroking.

Latencies of the responses to the cutaneous stimulation

The histograms in Fig. 2 show the initial spike latencies of the responses of candidate units (group I, II, III) to the hunting, cutaneous tapping and electrical stimuli. Two peaks are obvious in all histograms. Because the fast and late peaks corresponded to the latencies of the early short burst and the late discharges in responses of the candidate units, respectively, these responses should be divided at the trough into two groups. We adopted, for convenience, 63 msec latency as the dividing point between the fast and late groups of responses.

The mean latency of the fast group responses is shown on the left side of each histogram, and that of the late group is shown on the right side. The mean latencies of the fast group responses to the stimulation of the forelegs were significantly shorter than that for the responses to stimulation of the hindlegs (Fig. 2, B, C, D, and E. $p < 0.005$). There was no significant difference between the mean latencies of the fast group responses to the hunting and to the bilateral hindleg stimulation (Fig. 2, A, C and E. $p > 0.2$). There was no significant difference between the mean latencies of the fast group responses to stimulation of the contra- and ipsilateral forelegs (Fig. 2, B and D. $p > 0.5$) and between the latencies to stimulation of the contra- and ipsilateral hindlegs (Fig. 2, C and E. $p > 0.2$). There was no significant difference in the mean latencies of the late group responses ($p > 0.05$).

DISCUSSION

The responses to mechanical and electrical stimulation of the skin were tested on the candidate units of the pontine defecation reflex center. Most units (71%) of 168 candidate units tested responded to the tapping and/or to the

electrical skin stimulation, and 37% of 115 candidate units responded to the stroking (Table 1, 2). This result supports the assumption that the pontine defecation reflex center mediates the reflex effect of cutaneous stimulation on colonic motility.

Roles of group I–IV units

Stimulation of the pontine defecation reflex center elicits violent discharges on the rectal parasympathetic nerve and propulsive contractions of the colon[5]; therefore the pontine reticular cells, which mediate reflex enhancement of colonic motility, should be activated by the afferents of the reflex, but should be inhibited or not influenced by other kinds of afferents such as those that cause reflex inhibition of colonic motility.

Gentle stroking of the skin of dogs usually causes reflex discharges of the rectal parasympathetic fibers followed by an enhancement of colonic motility, while noxious cutaneous stimulation usually causes an inhibition of outflow in the fibers and colonic motility (Okada and Fukai, unpublished result). The tapping and electrical stimulation were in the noxious range. The cells of the pontine defecation reflex center, which mediate the enhancing effect of the stroking, should be excited by the stimulation and inhibited or not influenced by the noxious stimulation. The behavior of only three (one of group II and two of group III) of 63 candidate units fitted the requirements well, therefore these three units may be the mediator of the enhancing effect of the cutaneous–colonic reflex.

This number is possibly too small to mediate the cutaneous–colonic reflex. Because the tapping and electrical stimulation were in the noxious range, Aδ and C fibers can be activated as well as Aβ fibers. Hence, the afferent fibers responding to the stroking should be activated by the noxious stimulation. As result, the candidate units that received only the projection of the sensory fibers for stroking should be activated by the noxious stimulation. The candidate units activated by both kinds of stimuli were 21 (32%) of the 65 units tested. This percentage of the candidate units may be enough to mediate the enhancing effect of the cutaneous-colonic reflex. This result confirms that the pontine defecation reflex center mediates the cutaneous-colonic reflex.

Some group IV units are reticulo-spinal cells whose axons extend to the lumbo-sacral cord[1]. This group of units is inhibited by activity of the afferent pathway of the recto-rectal[1,3], the ano-colonic[4,6] and the gastro-colonic[6,7] reflexes, which elicit a violent discharge of the pelvic parasympathetic fibers and a propulsive contraction of the distal colon[6]. From these properties of group IV units, it seems that some of this group of cells have an inhibitory effect on the sacral defecation reflex center. If this is the case, the units should be activated by the afferent impulses that elicit an inhibition of the sacral parasympathetic outflow and colonic motility via the pontine defecation reflex center. Twenty-two units of 54 group IV units behaved in accord with this. This also confirms that the pontine defecation reflex center mediates the cutaneous-colonic reflex.

Segundo et al.[8] (1967) performed a systematical survey of the responses and receptive fields of reticular cells in the medulla oblongata of cats to cutaneous stimulation and calculated the ratios of cells responding differently to the stimulation ; however, such systematical surveys have not been done on the pontine reticular cells. Therefore, there is no data available for comparison with the present results to clarify the specificity of the candidate units selected by the hunting stimulation.

REFERENCES

1) Fukuda, H., Fukai, K., Yamane, M. and Okada, H. : Pontine reticular unit responses to pelvic nerve and colonic mechanical stimulation in the dog. Brain Res. **207** : 59–71, 1981
2) Okada, H., Fukuda, H. and Yamane, M. : The efferent activity in the pelvic nerve during the recto-rectal reflex of the dog. Auton. Nerv. System **12** : 278–287, 1975 (in Japanese)
3) Okada, H., Fukuda, H. and Yamane, M. : On the localization of the pontine defecation reflex center of the dog. Auton. Nerv. System **13** : 24–31, 1976 (in Japanese)
4) Okada, H., Yamane, M., Fukuda, H. and Fukai, K. : The ano-colonic reflex and parasympathetic outflow to the colon in the dog. Auton. Nerv. System **14** : 267–276, 1977 (in Japanese)
5) Fukuda, H., Fukai, K., Yamane, M. and Okada, H. : On the activity of the pontine defecation reflex center. J. Physiol. Soc. Japan **39** : 143, 1977 (in Japanese)
6) Fukuda, H. and Fukai, K. : Convergence of visceral afferents on candidate units for the pontine defecation reflex center of the dog. Jpn. J. Physiol. **32**, 1982 (in press)
7) Okada, H. and Fukai, K. : A role of the pontine defecation reflex center on the gastro-colonic reflex in dogs. Auton. Nerv. System **16** : 230–236, 1979 (in Japanese)
8) Segundo, J. P., Takenaka, T. and Encabo, H. : Somatic sensory properties of bulbar reticular neurons. J. Neurophysiol. **30** : 1221–1238, 1967