

Crossed Reflex Actions by Low Threshold Muscle Afferents

A number of crossed spinal reflex actions are known to exist¹ but there have been few attempts to analyse them in terms of the contributions from various somatic afferent systems. Crossed effects by volleys in high threshold muscle afferents have been reported² and PERL has described certain actions by single volleys in the large muscle afferents (group I)³. He found that a group I volley from knee flexors or extensors evokes inhibition followed by facilitation in motoneurons of corresponding contralateral muscles and, less consistently, a reciprocal pattern in motoneurons of their antagonists. Crossed effects on adequate activations of muscle receptors were assumed to be mediated by group I afferents⁴.

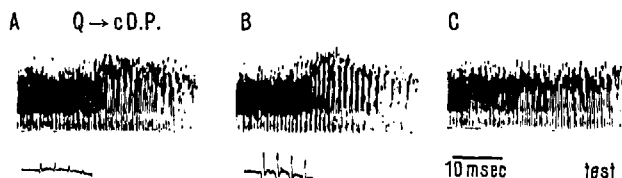
Cats made spinal by high lumbar section about two weeks before the final experiment, were used in the present investigation, because it was found that crossed actions from low threshold afferents were easiest elicited in these preparations. Even so, single conditioning maximal group I volleys hardly ever influenced contralateral monosynaptic test reflexes (whereas volleys in high threshold muscle afferents had pronounced effects). On the other hand, if a train of conditioning volleys were given, considerable contralateral group I effects were observed. This is illustrated in the Figure where the monosynaptic test reflex from the DP (deep peroneal = *tibialis anterior* + *extensor digitorum longus*) nerve is conditioned by a train of group I volleys from the contralateral quadriceps nerve. Each record consists of many (about 150) superimposed traces of monosynaptic reflex discharges scattered across the screen, while the conditioning stimuli are given at a fixed interval after the sweep start. The unconditioned monosynaptic test discharge is shown alone in record C. A clearcut crossed action appears already at a stimulus strength of 1.7 times threshold (A) and the action increases when the stimulus strength is raised to 2.75 times threshold (B), which is just submaximal group I. In excitable preparations, it was often sufficient to use two conditioning volleys to evoke crossed actions, otherwise 3–6 were needed. By increasing the number of conditioning stimuli the effect increased markedly. On repetitive stimulation at constant strength the successive volleys gradually decreased. When high range group I stimulation was used, monophasic recording of the incoming volley from dissected dorsal root-filaments was often made afterwards; and it was proved that the effects observed were evoked at stimulus strengths subliminal to group II fibres. In special control experiments it was established that at frequencies used there was no temporal summation of subthreshold stimuli in group II fibres, in other words a train of stimuli did not activate fibres not activated by the first stimulus.

In the Figure it should also be noted that a stimulus activating only about one fifth of the group I fibres was sufficient to evoke crossed actions (A). Such low threshold activation was also frequently observed between extensors. The stimulus strength was regularly increased with fine gradations which resulted merely in an increased effect. There was never any evidence of dual crossed group I actions (cf. Figure where records A and B are chosen from a series with increasing stimulus strength).

The most striking and constant feature of the crossed group I pattern is the prominent excitatory action between extensors (*quadriceps*, *gastrocnemius-soleus*, *plantaris* and *flexor digitorum longus*). There was never any inhibitory action from extensors to any contralateral extensor nucleus. From flexors investigated, facilitation was found from DP to contralateral extensor nuclei, while

BSt (*posterior biceps-semitendinosus*) seldom evoked any group I effect, but inhibition was observed once to quadriceps.

As regards the receptiveness of flexor nuclei, there were differences between BSt and DP. Group I volleys from extensors usually facilitated DP, whereas actions to BSt were less frequent. Between flexors group I, facilitation from DP to DP was observed but never any effect from BSt to BSt.



Upper records consist of superimposed traces of many monosynaptic reflex discharges evoked from the nerve to the deep peroneal muscles (*tibialis anterior* + *extensor digitorum longus*) and recorded in the ventral root. At each sweep the reflex discharge is automatically delayed so as to traverse the screen and this procedure is repeated 4 times. The distribution of reflex discharges is not uniform, the density decreases in the end of the record. The unconditioned test reflexes are shown in C, and in A and B the actions of a train of conditioning volleys from the contralateral quadriceps nerve evoked at a stimulus strength of 1.7 respectively 2.75 times threshold for the nerve. Lower traces show the conditioning volleys from the quadriceps nerve recorded in the dorsal root entry zone.

To summarize. The use of repetitive stimulation has proved valuable for disclosing crossed group I connections and a description of their pattern has been given. On the other hand, with repetitive stimulation it is more difficult to separate contributions from the fast and slow component of the group I volley, and so far it has not been possible to differentiate conclusively between actions evoked from large muscle spindle afferents and Golgi tendon organ afferents.

Zusammenfassung. Bei chronisch spinalen Katzen wurde mit Hilfe tetanischer Reizung festgestellt, dass grosse afferente, von Muskeln kommende Fasern gekreuzte Wirkungen auf monosynaptische Reflexe ausüben. Die Ausdehnung der Effekte wird beschrieben.

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October 12, 1960.

¹ C. S. SHERRINGTON, Quart. J. exp. Physiol. 2, 109 (1909).

² E. R. PERL, J. Neurophysiol. 21, 101 (1958). – B. HOLMQVIST, Exper. 16, 459 (1960).

³ E. R. PERL, J. Neurophysiol. 21, 101 (1958).

⁴ E. R. PERL, J. Physiol. 145, 193 (1959).

On the Mechanism of Increasing the Tuberculostatic Activity of Isoniazid in the Presence of 4-Aminobenzoic Acid

We have shown in previous communications^{1,2} that 4-aminobenzoic acid (PABA) increased *in vitro* the bacteriotropic activity of isoniazid on *M. tuberculosis*, whereas 2-aminobenzoic acid exerted an exactly opposite influence³.

¹ R. URBANČÍK, Amer. Rev. Tuberc. 76, 706 (1957).

² R. URBANČÍK, Amer. Rev. Tuberc. 78, 802 (1958).

³ Z. ŠIMÁNE and R. URBANČÍK, Exper. 16, 148 (1960).

The mechanism by which PABA increased the activity of isoniazid is still unknown. It was, however, suggested by KUBALA⁴ that this might have been explained by competitive acetylation of PABA instead of isoniazid, which was known to operate *in vivo*⁵ and undoubtedly could have occurred also in the conditions *in vitro*.

The reliability of this hypothesis was first checked in an experiment where the synergistic influence of both PABA and 4-acetamido-benzoic acid (Ac-PABA) on the isoniazid activity was compared.

The strains *Mycobacterium* 607 and H 37 Rv were maintained by serial passages on the surface of the Proskauer-Beck synthetic liquid medium without albumine or serum. This medium (pH 6.8) was distributed in 50.0 ml amounts in 250 ml Erlenmeyer flasks, to which isoniazid alone or with either PABA or Ac-PABA was added, and seeded with one loop of a 7-days old culture of the *M. 607* strain or a 14-days old culture of the H 37 Rv strain. Isoniazid was sinter-sterilized and added aseptically to the medium, PABA and Ac-PABA were added to the medium before autoclaving. All experiments were run in four parallel

chromatography. The chromatograms were run ascendingly on No. 3 Whatman paper with use of *n*-propanol, water, and ammonia solvent (50 : 38 : 12). Ac-PABA was hydrolysed on the paper by spraying with 10% hydrochloric acid and exposing it for 10 min over boiling water. The spots were detected by dipping the paper into the Ehrlich reagent with acetone⁶.

Our attempts to demonstrate Ac-PABA in culture filtrates of both strains remained, however, unsuccessful. Thus we may conclude that under the conditions of our experiment PABA is not acetylated by either strain *in vitro*, and the competitive acetylation hypothesis of increasing the bacteriostatic effect of isoniazid in the presence of PABA can hardly be maintained.

Our experiments, however, have shown that Ac-PABA does not increase the effect of isoniazid on either of the strains used. Thus we may suggest that *in vivo*, where acetylation of PABA undoubtedly takes place, the combination of isoniazid and PABA will probably be no more effective than isoniazid alone, except perhaps with very high doses of PABA. This suggestion has been verified⁷.

Table I

Drug	Concentration in µg/ml	Dry weight of bacteria in g ^{a, b}
Control	—	0.5424
PABA	200	0.5290
Ac-PABA	260 ^c	0.5513
Isoniazid	1	0.0692
Isoniazid + PABA	1 + 200	0.0316
Isoniazid + Ac-PABA	1 + 260	0.0888

^a Strain *Mycobacterium* 607.
^b Mean value from four parallel flasks.
^c Equimolar amount to 200 µg of PABA.

Table II

Drug	Concentration in µg/ml	Dry weight of bacteria in g ^{a, b}
Control	—	0.2933
PABA	200	0.2849
Ac-PABA	260 ^c	0.2906
Isoniazid	0.05	0.0581
Isoniazid + PABA	0.05 + 200	0.0333
Isoniazid + Ac-PABA	0.05 + 260	0.0599

^a Strain H 37 Rv.
^b Mean value from four parallel flasks.
^c Equimolar amount to 200 µg of PABA.

flasks. After 7 days incubation at 37°C for the *M. 607* strain or 14 days incubation for the H 37 Rv strain all cultures were autoclaved and the bacterial mass was separated by filtration from the medium. Further description of the method may be found elsewhere¹. Concentrations of the drugs as well as results of the experiments are summarized in Table I and II.

It may be seen from both Tables that, in both the *M. 607* or the H 37 Rv strain, Ac-PABA was ineffective in increasing the tuberculostatic properties of isoniazid, whereas PABA showed the previously described synergistic influence on the isoniazid activity. These results appear to offer some evidence in favour of the afore mentioned competitive acetylation hypothesis. Nevertheless, a direct proof of acetylation of either isoniazid or PABA by mycobacterial cultures *in vitro* was definitely needed. It is difficult to trace acetylated isoniazid under the conditions of our experiments. We attempted therefore to prove the formation of Ac-PABA from PABA in mycobacterial cultures *in vitro*.

The Proskauer-Beck medium (pH 6.8), to which 200.0 µg of PABA per ml were added before autoclaving was distributed in Erlenmeyer flasks and seeded as described above. After incubation for the afore-mentioned time intervals, the cultures of both strains were autoclaved and the bacterial mass was separated by filtration from the medium. The filtrates, each time from a control medium and from the medium with PABA, were further investigated as to their content of Ac-PABA by paper

Zusammenfassung. 4-Acetaminobenzoessäure hat *in vitro* im Unterschied zu 4-Aminobenzoessäure, keinen günstigen Einfluss auf die antituberkulotische Wirkung von Isoniazid. Bei den Stämmen *Mycobacterium* 607 und H 37 Rv wurde jedoch chromatographisch keine Bildung acetylierter 4-Aminobenzoessäure nachgewiesen.

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September 28, 1960.*

⁴ E. KUBALA, Amer. Rev. Tuberc. 78, 949 (1958).

⁵ J. C. BELL, D. K. RIEMENSNIER, and R. S. MITCHELL, Amer. Rev. Tuberc. 76, 152 (1957).

⁶ J. B. JEPSON, Lancet ii, 1009 (1955).

⁷ R. URBANČÍK, Z. ŠIMÁNEŠ, P. KRAUS, and I. REIL, Exper. 17, 84 (1961).

Isoniazid and 4-Aminobenzoic Acid in the Treatment of Murine Tuberculosis¹

As shown previously, 4-aminobenzoic acid (PABA) increases the tuberculostatic activity of isoniazid *in vitro*². It was now of interest to investigate the effect of isoniazid combined with PABA in the conditions *in vivo*.

¹ Part of these experiments was performed in the Institute of Microbiology, University of Brno.

² R. URBANČÍK, Amer. Rev. Tuberc. 76, 706 (1957).