UC Irvine

UC Irvine Previously Published Works

Title

ATP release from dorsal spinal cord synaptosomes: characterization and neuronal origin.

Permalink

<https://escholarship.org/uc/item/8rx439zv>

Journal

Brain research, 610(1)

ISSN

0006-8993

Authors

Sawynok, J
Downie, JW
Reid, AR
[et al.](#)

Publication Date

1993-04-01

DOI

10.1016/0006-8993(93)91213-c

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

ATP release from dorsal spinal cord synaptosomes: characterization and neuronal origin

Jana Sawynok, John W. Downie, Allison R. Reid, Catherine M. Cahill and Thomas D. White

Department of Pharmacology, Dalhousie University, Halifax, NS (Canada)

(Accepted 24 November 1992)

Key words: Adenosine 5'-triphosphate; Spinal cord; Synaptosome; Capsaicin; Rhizotomy; 6-Hydroxydopamine

The present study determined the Ca^{2+} -dependence of the release of adenosine 5'-triphosphate (ATP) from dorsal spinal cord synaptosomes evoked by depolarization with K^+ and capsaicin, and the effect of intrathecal capsaicin pretreatment, dorsal rhizotomy and intrathecal pretreatment with 6-hydroxydopamine on such release. Release of ATP evoked by K^+ was Ca^{2+} -dependent, while that evoked by capsaicin was Ca^{2+} -independent. Capsaicin pretreatment (60 μg , 7 days), which lesions small diameter afferents, did not alter release of ATP evoked by either K^+ or capsaicin. Dorsal rhizotomy, which lesions all afferents, produced a significant reduction in the amount of ATP released from the rhizotomized side compared to the intact side. Pretreatment with 6-hydroxydopamine (100 μg , 7 days) to destroy adrenergic nerve terminals, markedly reduced spinal cord noradrenaline levels, but did not alter the K^+ -evoked release of ATP. These results suggest that some K^+ -evoked release of ATP could originate from large but not small diameter afferent nerve terminals in the spinal cord. ATP does not appear to originate from small diameter afferents as, although ATP is released by *in vitro* exposure to capsaicin, such release occurs only at high concentrations, release is Ca^{2+} -independent and it is unaltered by pretreatment with capsaicin. The bulk of the ATP released from the spinal cord does not originate from descending noradrenergic nerve terminals.

INTRODUCTION

Adenosine and ATP modulate neuronal activity in the central nervous system²². ATP may exert direct actions by activating P_2 purinoreceptors or indirect actions by activating P_1 purinoreceptors following conversion to adenosine⁵. 5'-Nucleotidase, which hydrolyses 5'-AMP to adenosine, is distributed throughout the central nervous system¹⁷ as well as in the spinal cord¹⁹. Additional actions of ATP also may occur at P_1 purinoreceptors without prior conversion to adenosine²⁵. Within the spinal cord, adenosine has inhibitory influences on the transmission of nociceptive information³⁰. There is increasing evidence that ATP also may play a role in sensory transmission. Such a role was first considered by Holton¹² who demonstrated that ATP was released by antidromic stimulation of sensory nerves. In an *in vitro* preparation, ATP has been shown to excite a subpopulation of dorsal horn neurons in culture¹⁴, while *in vivo*, ATP selectively excites non-nociceptive neurons in the dorsal spinal cord^{8,27}

(but see ref. 26). Interestingly, the predominant effect of ATP on wide dynamic range neurons is a biphasic excitation followed by a depression of activity²⁷. The reduction in activity appears due to activation of P_1 purinoreceptors as it is blocked by methylxanthines²⁷. Inhibition of nociceptive neurons in the spinal cord evoked by peripherally applied mechanical vibration is reduced by a methylxanthine adenosine receptor antagonist, and it has been proposed that the effects of vibration are due to activation of large diameter afferents, release of ATP and subsequent conversion to adenosine which could then regulate the activity of nociceptive neurons²⁸.

In neurochemical experiments, ATP has been shown to be released from spinal cord synaptosomes by depolarization with K^+ ⁴⁰, veratrine alkaloids^{40,44} and capsaicin³². In all cases, ATP release from dorsal spinal cord synaptosomes exceeded that from ventral spinal cord synaptosomes, which is consistent with a role for ATP in sensory function. In the present study, we have characterized the release of ATP from dorsal spinal

cord synaptosomes and examined some possible neuronal origins for ATP with specific reference to a possible role in nociceptive function. A number of issues are addressed. (a) *Ca²⁺-dependence of release.* K⁺-evoked release of ATP is Ca²⁺-dependent, while veratridine-evoked release is augmented in Ca²⁺-free medium⁴⁰. Capsaicin generally exerts selective effects on small-diameter sensory neurons¹³. It binds to a receptor site and activates a non-selective cation channel which allows the entry of Ca²⁺ into neurons^{4,13,42}. The Ca²⁺-dependence of capsaicin-evoked release of ATP has not been determined. (b) *The effect of pretreatment with capsaicin on ATP release evoked by K⁺ and capsaicin.* In vitro exposure to capsaicin releases adenosine (which originates as nucleotide) from the dorsal spinal cord, and this originates from capsaicin-sensitive afferents³². In determining the effect of capsaicin pretreatment on release of ATP, the aim was to determine whether ATP originates from capsaicin-sensitive afferents and could serve as a precursor for the adenosine released by in vitro exposure to capsaicin. (c) *The effect of dorsal rhizotomy on K⁺-evoked release of ATP.* If ATP originates from large diameter afferents, dorsal rhizotomy, which leads to degeneration of afferent nerves in the dorsal spinal cord¹⁰ and a marked depletion of peptides known to be contained in sensory afferents^{6,11,35}, should reduce release of ATP. (d) *The effect of the adrenergic neurotoxin 6-hydroxydopamine (6-OHDA) on ATP release from the dorsal spinal cord.* There is ample evidence for the co-storage and co-release of ATP with noradrenaline (NA) in the peripheral nervous system^{36,37}. The release of ATP following intrathecal pretreatment with 6-OHDA was examined to determine if ATP originates from noradrenergic nerve terminals arising from bulbospinal projection pathways.

MATERIALS AND METHODS

Animals

Experiments were performed on 56 male Sprague-Dawley rats (Charles River, Que., Canada) 275–350 g.

Preparation of synaptosomes

Synaptosomes were prepared from the dorsal spinal cord following decapitation. Tissue was homogenized in ice-cold sucrose buffered with HEPES (pH 7.4), and centrifuged at 1,000 × *g* for 10 min. The supernatant was further centrifuged at 12,000 × *g* for 20 min. The pellet was resuspended in ice-cold Krebs-Henseleit medium (containing NaCl 111 mM, KCl 4.7 mM, MgCl₂ 1.2 mM, CaCl₂ 1.8 mM, NaH₂PO₄ 1.2 mM, NaHCO₃ 26.2 mM, glucose 11 mM, gassed with 95% O₂/5% CO₂) and centrifuged at 20,000 × *g* for 30 min. The resulting crude P2 synaptosomal fraction was suspended in Krebs-Henseleit medium and incubated for 30 min at 37°C. Synaptosomes were recentrifuged, and resuspended in Krebs-Henseleit at 37°C to yield a final protein concentration of 0.8–1.5 mg protein/ml.

Quantitation of ATP release

Release of ATP evoked by K⁺ and capsaicin was determined directly using the firefly luciferin-luciferase assay as described previously³⁸. This involved addition of a 20 μl mixture of equal parts of D-luciferin (5 mg/ml) and crude firefly extract containing luciferase (50 mg FLE-50/2 ml) to 500 μl of the synaptosomal suspension in a cylindrical cuvette, mixing, and placement into a Chemiluminometer (Aminco). Reagents were injected in a volume of 10 μl. ATP release was quantitated by reference to standard amounts of ATP added to the cuvette after the treatment, and was expressed as the maximum molar concentration of ATP obtained per mg protein.

Ca²⁺-free experiments

For Ca²⁺-free experiments, synaptosomes were prepared in a Ca²⁺-free Krebs-Henseleit medium. Ca²⁺ (1.8 mM) or EGTA (1 mM, buffered to pH 7.4 with 1 N NaOH) was added to the cuvette. In these experiments, release was assessed from the dorsal lumbar and thoracic portions of the spinal cord. Two dorsal spinal cord sections were pooled for each experiment.

Intrathecal pretreatments

Rats were pretreated intrathecally (i.t.) with the neurotoxin capsaicin (60 μg/15 μl in 50% dimethylsulfoxide (DMSO)/saline) or vehicle, or 6-OHDA (100 μg/15 μl in 0.1% ascorbic acid) or vehicle. For these experiments, rats were anaesthetized with halothane and mounted in a stereotaxic apparatus. The skin and muscles overlying the cisterna were retracted, and a small hole made in the membrane. A catheter (PE 10 tubing) was inserted 7.5 cm into the spinal subarachnoid space to the lumbar level. Following drug injection, the catheter was allowed to remain in position for 5 (6-OHDA) or 10 (capsaicin) min prior to slow withdrawal. The muscles and skin were sutured, and the rat allowed to recover. Rats were treated postoperatively with Penlong G i.m. and 10 ml lactated Ringer's solution s.c. Release studies for both the capsaicin and 6-OHDA pretreatment experiments were performed 6–8 days following i.t. injections. In both cases, release was assessed from the dorsal lumbar-sacral spinal cord only to correspond to the site of delivery of the neurotoxin. Tissue from two rats was pooled for each experiment for the capsaicin experiment. Tissue from individual rats was assessed for the 6-OHDA experiment, and ATP release was determined in duplicate.

Following 6-OHDA pretreatment, the ventral lumbar-sacral and thoracic spinal cord was extracted in 0.1 N perchloric acid and assayed by high pressure liquid chromatography with electrochemical detection for NA content³². The corresponding dorsal lumbar segment was used to prepare synaptosomes and determine ATP release, and it was assumed that the depletion observed in adjacent tissue was comparable to that in the dorsal lumbar spinal cord.

Dorsal rhizotomies

Rats were anaesthetized with halothane and stabilized in a stereotaxic apparatus. A hemilaminectomy was performed on the left side from the T₁₁ to L₁ vertebrae. The dura was cut and dorsal roots corresponding to spinal segments T₁₃ to S₁ were identified under a dissecting microscope and transected. Care was taken not to damage blood vessels or spinal tissue. In sham rats, a hemilaminectomy was performed followed by opening of the dura, but the roots were not touched. The exposed spinal cord was covered with saline-moistened Gelfoam. The area was then sutured in two layers. Bupivacaine was injected into wound margins in muscle and skin. Rats were injected postoperatively with Penlong G^R i.m. and 10 ml lactated Ringers s.c. Ten rats received the rhizotomy and 10 rats received a sham surgery. Following surgery, individual rats were housed in the presence of a female rat to prevent autotomy³. Release studies were performed 14 days after surgery. At this time, the mean body weight of the rhizotomized rats was within 10% of the sham-operated controls. Following removal of the spinal cord and division into dorsal and ventral portions, the lumbar-sacral cord was hemisected into the rhizotomized and intact side. Tissue from two spinal cords was pooled, and ATP release determinations performed in duplicate.

Statistics

Results were analyzed using analysis of variance followed by the Student/Newman-Keuls test when multiple comparisons were made, or by the unpaired *t*-test when two comparisons were made.

Drugs and sources

The following agents were used: ATP (adenosine 5'-triphosphate, sodium salt), DMSO, EGTA (ethylene glycol-bis(β -aminoethyl ether) *N,N,N',N'*-tetraacetic acid), capsaicin (8-methyl-*N'*-vanillyl-6-nonenamide), 6-OHDA hydrochloride, ascorbic acid, D-luciferin, crude firefly extract (FLE-50) (Sigma Chemical Co., St. Louis, MO).

RESULTS

Ca^{2+} -dependence of ATP release

Exposure to elevated K^+ concentrations leads to the release of ATP from dorsal spinal cord synaptosomes⁴⁰.

Fig. 1. Release of ATP from dorsal spinal cord synaptosomes by K^+ , DMSO and capsaicin in the absence and presence of Ca^{2+} . Synaptosomes were prepared in Ca^{2+} -free Krebs–Henseleit medium (left panels), and $CaCl_2$ (central panels) and EGTA (right panels) added as required. All treatment and drug additions were in a volume of 10 μl . Release of ATP was quantitated by reference to standard doses of ATP added following the treatment.

The extent of release depends on the concentration of K^+ added (Fig. 3A). For Ca^{2+} -free experiments, synaptosomes were prepared in a Ca^{2+} -free Krebs–Henseleit medium. Release in the presence of a normal Ca^{2+} concentration (1.8 mM) was determined following restoration of normal Ca^{2+} levels by addition of $CaCl_2$ to the synaptosomal suspension. Addition of Ca^{2+} leads to some decline in the basal amount of light detected (Fig. 1, central panels). Addition of 36 mM K^+ evoked release of ATP under normal Ca^{2+} conditions (Fig. 1A, central panel, Fig. 2A). In the Ca^{2+} -free medium, there was a significant reduction in the amount of ATP released by K^+ (Fig. 1A, left panel; Fig. 2A). Following the addition of EGTA (1 mM) to the cuvette (which in itself enhanced the basal amount of light detected), K^+ no longer released ATP; in fact there was a decline in the basal amount of light emitted (Fig. 1A, right panel; Fig. 2A). Thus, in the presence of EGTA, the response to adding KCl now resembles the response seen following addition of NaCl³⁸, which probably represents a response to the enhanced tonicity of the medium.

Exposure of dorsal spinal cord synaptosomes to capsaicin in vitro produces a dose-dependent release of ATP (Fig. 3B) (see also ref. 32). The vehicle used for capsaicin (50% DMSO/saline; approximately 1% following addition to the cuvette) evoked a significant release of ATP (Fig. 1B, Fig. 3B). The release of ATP evoked by DMSO appears Ca^{2+} -dependent, as it is significantly reduced in the absence of Ca^{2+} and presence of EGTA (Fig. 1B, right panel; Fig. 2B). Doses of capsaicin $> 100 \mu M$ release ATP (Fig. 2B). Release of ATP evoked by capsaicin does not appear to be Ca^{2+} -dependent, as release is essentially unaltered in the Ca^{2+} -free medium containing EGTA. The small reduction in release seen with 100 μM capsaicin in Ca^{2+} -free medium containing EGTA (Fig. 2B) may reflect the Ca^{2+} -dependence of the DMSO component, and this is of less consequence at 150 μM capsaicin.

Effect of i.t. pretreatment with capsaicin on K^+ and capsaicin-evoked release of ATP

An i.t. pretreatment with capsaicin did not significantly alter either the K^+ or the capsaicin-evoked release of ATP from dorsal spinal cord synaptosomes (Fig. 3).

Effect of dorsal rhizotomy on K^+ evoked release of ATP

The K^+ -evoked release of ATP from the left side of the sham operated controls was the same as that from the right side. In rhizotomized animals, there was a significant reduction in the amount of ATP released from the rhizotomized (left) side compared to the intact (right) side, with release being 65% of that from

Fig. 2. Ca²⁺-dependent release of ATP from dorsal spinal cord synaptosomes by K⁺ but not by capsaicin. Hollow columns depict release in the normal Ca²⁺ medium, hatched columns release in the Ca²⁺-free medium, and cross-hatched columns release in the Ca²⁺-free medium containing EGTA. Values are mean $\pm$ S.E.M. for $n = 4$. * $P < 0.05$, *** $P < 0.001$ compared to release in the presence of 1.8 mM Ca²⁺.

the intact side (Fig. 4). Some of this difference may be due to an enhanced release in the intact side, as release from the rhizotomized side appears similar to that from both the left and right sides in the sham operated group. While mean protein values in synaptosomal suspensions prepared from the left vs. the right side were within 11% of each other in both groups, the mean protein value in the intact side of the rhizotomized animals was the lowest (SHAM left 1.12 ± 0.06 , right 1.01 ± 0.09 , RHIZ left 0.94 ± 0.04 , right 0.81 ± 0.03 mg protein/ml). This suggests that the tendency towards an enhanced release on the intact side may

potentially be due to the method of analysis. On the other hand, it should be noted that prior to correction for protein values, the K⁺-evoked release from the rhizotomized side was still significantly lower than the intact side in the same animals (fractional release 0.97 ± 0.11 compared to 1.33 ± 0.08 , $P < 0.02$).

Effect of i.t. pretreatment with 6-hydroxydopamine on the K⁺-evoked release of ATP

An i.t. pretreatment with 6-OHDA reduced NA levels in the ventral lumbar-sacral spinal cord by 85% and in the thoracic spinal cord by 78%. However,

Fig. 3. Effect of i.t. pretreatment with capsaicin (○, 60 µg, 7 days) or vehicle (●) on release of ATP from dorsal lumbar-sacral spinal cord synaptosomes by K⁺ and capsaicin. Values are mean $\pm$ S.E.M. for $n = 4$.

Fig. 4. Effect of dorsal rhizotomy (RHIZ) on the K⁺-evoked release of ATP from synaptosomes prepared from the left (L) or right (R) side of the lumbar-sacral spinal cord. Rhizotomies ($n = 10$) and sham surgeries ($n = 10$) were performed on the left side 14 days prior to the release experiment. * $P < 0.05$ ($n = 5$ per group).

release of ATP evoked by 24 mM K⁺ was not altered in the corresponding dorsal lumbar-sacral spinal cord (Fig. 5).

DISCUSSION

In this study, we have examined a number of aspects of ATP release from the dorsal spinal cord, with a particular emphasis on examining potential release from sensory afferents and bulbospinal noradrenergic pathways. K⁺-evoked release of ATP from dorsal spinal cord synaptosomes was shown to be Ca²⁺-dependent, as noted previously⁴⁰. K⁺-evoked release of ATP from synaptosomes prepared from whole brain or various brain regions similarly is Ca²⁺-dependent^{24,34,38}. Such release is consistent both with a vesicular origin of ATP (either as a sole or co-transmitter), and with some

form of Ca²⁺-dependent release which has yet to be defined⁴¹. In contrast, release of ATP evoked by capsaicin is not Ca²⁺-dependent. Capsaicin is known to bind to specific membrane receptors and subsequently to activate a non-selective cation channel to allow Ca²⁺ entry into neurons^{4,13,42}. Release of neuropeptides⁹ and adenosine³² evoked by capsaicin is Ca²⁺-dependent. However, the synaptosomal release of ATP evoked by capsaicin appears different from these processes in that it requires higher concentrations (> 50–100 μ M compared to 0.1–10 μ M) and is not Ca²⁺-dependent. Although capsaicin is generally regarded as having actions which are selective for small diameter afferent neurons, at higher concentrations capsaicin also may have actions on a range of other cell types¹³. These cell-non-selective actions are usually seen in the 100 μ M range^{2,33}, and may be due to changes in membrane fluidity¹⁸. It is likely that the present effect of capsaicin represents a cell-non-selective action of capsaicin on dorsal spinal synaptosomes. Accordingly, i.t. pretreatment with capsaicin, does not alter the capsaicin-evoked release of ATP from the dorsal spinal cord. This pretreatment causes degeneration of C type primary afferent neurons in the substantia gelatinosa²¹ and reduces the dorsal spinal cord contents of peptides contained in small diameter afferent neurons^{20,29,43}. Although capsaicin releases a nucleotide which is subsequently degraded to adenosine from capsaicin-sensitive afferents³², this nucleotide does not appear to be ATP. ATP release evoked by K⁺ also is not altered by the capsaicin pretreatment, suggesting that small diameter afferent fibres are not the source for ATP released from the dorsal spinal cord.

Fig. 5. Effect of i.t. pretreatment with 6-OHDA (100 μ g, 7 days) on (A) release of ATP from dorsal lumbar spinal cord synaptosomes (D-LSC), and (B) NA levels in the corresponding ventral lumbar spinal cord (V-LSC) and thoracic spinal cord (TSC). *** $P < 0.001$ ($n = 6$).

The present study demonstrates that dorsal rhizotomy produces some reduction in the release of ATP evoked by K^+ from dorsal spinal cord synaptosomes, as release from the rhizotomized side is significantly reduced compared to the intact side in the same animal. It should be noted that while some of the difference may appear to be accounted for by an increase in release from the intact side, this increase may reflect the method of expression of results which relies on protein levels. Alternatively, some change in the contralateral side may occur due to transneuronal degeneration. If the difference is real, some ATP release may originate from large diameter afferents. Dorsal rhizotomy causes neuronal degeneration in the dorsal horn of the spinal cord¹⁰ and induces a marked depletion in the levels of calcitonin gene-related peptide and substance P which are located in small diameter afferents^{6,11,15,35}. Although it is not clear whether large diameter afferents degenerate at the same rate as small diameter afferents, degenerative changes are apparent 4 days following rhizotomy¹⁰, while changes in peptide content are well developed at the 14-day interval examined in this study³⁵.

A previous study attempted to determine if ATP release originated from sensory afferents in the spinal cord by examining the effect of peripheral nerve lesions on ATP release from the spinal cord⁴⁰. No significant alteration in release was observed following such lesions. Different central consequences to peripheral nerve lesions and rhizotomy have been noted previously. Thus, although peripheral nerve lesions reduce the content of substance P and calcitonin gene-related peptide in the dorsal horn, the dorsal root ganglion cell body and the peripheral nerve^{15,35}, the extent of the reduction is less than that induced by rhizotomy, suggesting a less complete degree of central degeneration following the peripheral lesion. Accordingly, some changes seen following rhizotomy, such as a decrease in opioid receptor binding, are not seen following peripheral nerve lesions¹⁵.

In the sympathetic nervous system, ATP is co-stored with NA in sympathetic neurons⁷. There is considerable evidence from a variety of tissues that ATP and NA are released and contribute to the response seen following sympathetic nerve stimulation^{36,37}. 6-OHDA has been shown to diminish the release of ATP from the guinea pig ileum^{1,39} indicating that ATP does originate from sympathetic neurons. In the present study, 6-OHDA did not alter the release of ATP from the dorsal spinal cord. This suggests that the bulk of the ATP released from this preparation does not originate from bulbospinal noradrenergic pathways, but does not preclude the possibility of corelease of a limited pool

of ATP from such a source. The observation that spinal transection does not alter veratridine-evoked release of ATP from the dorsal spinal cord⁴⁴ is in agreement with our results. It has been clearly established that 6-OHDA is neurotoxic, and causes degeneration of adrenergic nerve terminals¹⁶, and the dose of 6-OHDA used was clearly sufficient to produce a substantial depletion of NA in tissue adjacent to that from which the release was determined. Our result with 6-OHDA is thus more similar to findings with brain synaptosomes where 6-OHDA pretreatment did not alter ATP release²³ than to findings in the peripheral nervous system (see above).

In summary, the present study demonstrates that dorsal rhizotomy may produce some reduction in ATP release from the dorsal spinal cord. In view of the lack of effect of pretreatment with capsaicin, which causes degeneration of small-diameter afferent neurons, some of this ATP could originate from large-diameter afferent fibres. This observation would be consistent with the proposal by Salter and Henry²⁸ that ATP can be released from large-diameter afferent fibres by physiological manipulation. The consequence of such release appears to be modulation of nociceptive transmission in the dorsal horn of the spinal cord via P_1 purinoreceptors^{27,28,30}. Although capsaicin also can release ATP from the spinal cord, this release appears to occur from non-sensory neurons as it occurs only at high concentrations, is Ca^{2+} -independent and is not sensitive to a prior i.t. pretreatment with capsaicin. Thus, ATP release does not appear to originate from small-diameter afferent fibres in the spinal cord. This is in contrast to other purines (adenosine and unidentified nucleotides which are converted to adenosine) which do appear to originate from capsaicin-sensitive afferents^{31,32}.

Acknowledgements This work was supported by a grant to J.S. and T.D.W. from the Medical Research Council of Canada.

REFERENCES

- 1 Al-Humayyd, M. and White, T.D., Adrenergic and possible nonadrenergic sources of adenosine 5'-triphosphate release from nerve varicosities isolated from ileal myenteric plexus, *J. Pharmacol. Exp. Ther.*, 233 (1985) 796–800.
- 2 Barthó, L., Pethő, G., Antal, A., Holzer, P. and Szolcsányi, J., Two types of relaxation due to capsaicin in the guinea-pig isolated ileum, *Neurosci. Lett.*, 81 (1987) 146–150.
- 3 Berman, D. and Rodin, B.E., The influence of housing condition on autotomy following dorsal rhizotomy in rats, *Pain*, 13 (1982) 307–311.
- 4 Bevan, S. and Szolcsányi, J., Sensory neuron-specific actions of capsaicin: mechanisms and applications, *Trends Pharmacol. Sci.*, 11 (1990) 330–333.
- 5 Burnstock, G., A basis for distinguishing two types of purinergic receptor. In R.W. Straub and L. Bolis, *Cell Membrane Receptors*

- for *Drugs and Hormones: a Multidisciplinary Approach*, Raven, New York, 1978, pp. 107–118.
- 6 Chung, K., Lee, W.T. and Carlton, S.M., The effects of dorsal rhizotomy and spinal cord isolation on calcitonin gene-related peptide-labelled terminals in the rat lumbar dorsal horn, *Neurosci. Lett.*, 90 (1988) 27–32.
 - 7 Fried, G., Small noradrenergic storage vesicles isolated from rat vas deferens—biochemical and morphological characterization, *Acta Physiol. Scand. Suppl.*, 493 (1980) 1–35.
 - 8 Fyffe, R.E.W. and Perl, E.R., Is ATP a central synaptic mediator for certain primary afferent fibres from mammalian skin? *Proc. Natl. Acad. Sci. USA*, 81 (1984) 6890–6893.
 - 9 Gamse, R., Molnar, A. and Lembeck, F., Substance P release from spinal cord slices by capsaicin, *Life Sci.*, 25 (1979) 629–636.
 - 10 Heimar, L. and Wall, P.D., The dorsal root distribution to the substantia gelatinosa of the rat with a note on the distribution in the cat, *Exp. Brain Res.*, 6 (1968) 89–99.
 - 11 Hökfelt, T., Kellerth, J.O., Nilsson, G. and Pernow, B., Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurones, *Brain Res.*, 100 (1975) 235–252.
 - 12 Holton, P., The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves, *J. Physiol.*, 145 (1959) 494–504.
 - 13 Holzer, P., Capsaicin: Cellular targets, mechanisms of action and selectivity for thin sensory neurons, *Pharmacol. Rev.*, 43 (1991) 143–201.
 - 14 Jahr, C.E. and Jessell, T.M., ATP excites a subpopulation of rat dorsal horn neurones, *Nature*, 304 (1983) 730–733.
 - 15 Jessell, T., Tsunoo, A., Kanazawa, I. and Otsuka, M., Substance P: depletion in the dorsal horn of rat spinal cord after sections of the peripheral processes of primary sensory neurons, *Brain Res.*, 168 (1979) 247–259.
 - 16 Jonsson, G., Chemical neurotoxins as denervation tools in neurobiology, *Annu. Rev. Neurosci.*, 3 (1980) 169–187.
 - 17 Kreutzberg, G.W., Heymann, D. and Reddington, M., 5'-Nucleotidase in the nervous system. In G.W. Kreutzberg (Ed.), *Cellular Biology of Ectoenzymes*, Springer-Verlag, Berlin, 1986, pp. 147–164.
 - 18 Meddings, J.B., Hogaboam, C.M., Tran, K. and Reynolds, J.D., Capsaicin effects on non-neural plasma membranes, *Biochim. Biophys. Acta*, 1070 (1991) 43–50.
 - 19 Nagy, J.I. and Daddona, P.E., Anatomical and cytochemical relationships of adenosine deaminase-containing primary afferent neurons in the rat, *Neuroscience*, 3 (1985) 799–813.
 - 20 Nagy, J.I., Emson, P.C. and Iversen, L.L., A re-evaluation of the neurochemical and antinociceptive effects of intrathecal capsaicin in the rat, *Brain Res.*, 211 (1981) 497–502.
 - 21 Palermo, N.N., Brown, H.K. and Smith, D.L., Selective neurotoxic action of capsaicin on glomerular C-type terminals in rat substantia gelatinosa, *Brain Res.*, 208 (1981) 506–510.
 - 22 Phillis, J.W. and Wu, P.H., The role of adenosine and its nucleotides in central synaptic function, *Prog. Neurobiol.*, 16 (1981) 187–239.
 - 23 Potter, P.E. and White, T.D., Lack of effect of 6-hydroxydopamine pretreatment on depolarization-induced release of ATP from rat brain synaptosomes, *Eur. J. Pharmacol.*, 80 (1982) 143–147.
 - 24 Richardson, P.J. and Brown, S.J., ATP release from affinity-purified rat cholinergic nerve terminals, *J. Neurochem.*, 48 (1987) 622–630.
 - 25 Rubino, A., Amerini, S., Ledda, F. and Mantelli, L., ATP modulates the efferent function of capsaicin-sensitive neurones in guinea-pig isolated atria, *Br. J. Pharmacol.*, 105 (1992) 516–520.
 - 26 Salt, T.E. and Hill, R.G., Excitation of single sensory neurones in the rat caudal trigeminal nucleus by iontophoretically applied adenosine 5'-triphosphate, *Neurosci. Lett.*, 35 (1983) 53–57.
 - 27 Salter, M.W. and Henry, J.L., Effects of adenosine 5'-monophosphate and adenosine 5'-triphosphate on functionally identified units in the cat spinal dorsal horn. Evidence for a differential effect of adenosine 5'-triphosphate on nociceptive vs non-nociceptive units, *Neuroscience*, 3 (1985) 815–825.
 - 28 Salter, M.W. and Henry, J.L., Evidence that adenosine mediates the depression of spinal dorsal horn neurons induced by peripheral vibration in the cat, *Neuroscience*, 22 (1987) 631–650.
 - 29 Sawynok, J., Reid, A. and Nance, D., Spinal antinociception by adenosine analogs and morphine after intrathecal administration of the neurotoxins capsaicin, 6-hydroxydopamine and 5,7-dihydroxytryptamine, *J. Pharmacol. Exp. Ther.*, 258 (1991) 370–380.
 - 30 Sawynok, J. and Sweeney, M.I., The role of purines in nociception, *Neuroscience*, 32 (1989) 557–569.
 - 31 Sweeney, M., White, T. and Sawynok, J., 5-Hydroxytryptamine releases adenosine from primary afferent nerve terminals in the spinal cord, *Brain Res.*, 462 (1988) 346–349.
 - 32 Sweeney, M.I., White, T.D. and Sawynok, J., Morphine, capsaicin and K^+ release purines from capsaicin-sensitive primary afferent nerve terminals in the spinal cord, *J. Pharmacol. Exp. Ther.*, 248 (1989) 447–454.
 - 33 Szolcsányi, J. and Barthó, L., New type of nerve mediated cholinergic contractions of the guinea-pig small intestine and its selective blockade by capsaicin, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 305 (1978) 83–90.
 - 34 Terrian, D.M., Hernandez, P.G., Rea, M.A. and Peters, R.I., ATP release, adenosine formation, and modulation of dynorphin and glutamic acid release by adenosine analogues in rat hippocampal mossy fiber synaptosomes, *J. Neurochem.*, 53 (1989) 1390–1399.
 - 35 Villar, M.J., Wiesenfeld-Hallin, Z., Xu, X.-J., Theodorsson, E., Emson, P.C. and Hökfelt, T., Further studies on galanin-, substance P-, and CGRP-like immunoreactivities in primary sensory neurons and spinal cord: effects of dorsal rhizotomies and sciatic nerve lesions, *Exp. Neurol.*, 112 (1991) 29–39.
 - 36 von Kügelen, I. and Starke, K., Noradrenaline-ATP co-transmission in the sympathetic nervous system, *Trends Pharmacol. Sci.*, 12 (1991) 319–324.
 - 37 Westfall, D.P., Dalziel, H.H. and Forsyth, K.M., ATP as a neurotransmitter, cotransmitter, and neuromodulator. In J.W. Phillis (Ed.), *Adenosine and Adenine Nucleotides as Regulators of Cellular Function*, CRC Press, Boca Raton, FL, 1991, pp. 295–305.
 - 38 White, T.D., Release of ATP from a synaptosomal preparation by elevated extracellular K^+ and by veratridine, *J. Neurochem.*, 30 (1978) 329–336.
 - 39 White, T.D. and Al-Humayyd, M., Acetylcholine releases ATP from varicosities isolated from guinea pig myenteric plexus, *J. Neurochem.*, 40 (1983) 1069–1075.
 - 40 White, T.D., Downie, J.W. and Leslie, R.A., Characteristics of K^+ - and veratridine-induced release of ATP from synaptosomes prepared from dorsal and ventral spinal cord, *Brain Res.*, 334 (1985) 372–374.
 - 41 White, T.D. and MacDonald, W.F., Neuronal release of ATP and adenosine, *Ann. NY Acad. Sci.*, 603 (1990) 287–299.
 - 42 Wood, J.N., Winter, J., James, I.F., Rang, H.P., Yeats, J. and Bevan, S., Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture, *J. Neurosci.*, 8 (1988) 3208–3220.
 - 43 Yaksh, T.L., Farb, D.H., Leeman, S.E. and Jessell, T.M., Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia, *Science*, 206 (1979) 481–483.
 - 44 Yoshioka, K. and Jessell, T.M., ATP release from the dorsal horn of rat spinal cord, *Soc. Neurosci. Abstr.*, 10 (1984) 993.